IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Peter Bennett Duff Whyte	**Examiner**:	Deborah K Ware
Serial No.:	09/702,037	**Art Unit:**	1651
Filed:	October 27, 2000	**Docket:**	U013032-6

For: A FOOD COMPOSITION AND METHOD OF USING SAME

Confirmation No: 8344

Commissioner for Patents
Alexandria, VA 22313-1450

DECLARATION OF Jonathan Buckley UNDER 37 C.F.R. §1.132

Sir:

I, Jonathan Buckley, hereby declare as follows:

1. I am. an Associate Professor and Deputy Director of the Nutritional Physiology Research Centre and Co-Director of the Australian Technology Network Centre for Metabolic Fitness at the University of South Australia, Frome Road, Adelaide, South Australia 5000.

2. I hold a Doctorate Degree in Physiology.

3. In 1998 I was a Lecturer in Exercise Biochemistry and Physiology in the School of Physical Education Exercise and Sport Studies at the University of South Australia.

4. A true and correct copy of my curriculum vitae is attached hereto as **Exhibit A**.

5. I have carefully reviewed the present patent application, Serial No. 09/702,037 ("the '037 application") and have read the Official Action dated August 8, 2006. In that action

the examiner has made assertions that improved body composition and condition is achieved by the presence of IGF-1 levels, administered by bovine colostrum via the oral route.

6. However, it is unlikely that the IGF-1 contained in the bovine colostrum itself is the source of the elevation in circulating IGF-1 which has been associated with the consumption of some forms of bovine colostrum (eg Bioenervi™) (5) since the quantity of IGF-1 contained in these colostrum products is not sufficient to account for the magnitude of increase in circulating IGF-1 reported.

7. The following calculations show that the direct absorption of IGF-1 from orally administered bovine colostrum (Bioenervi™) could not fully account for all of the reported increase in circulating IGF-1 concentration that has been reported previously.

8. According to Mero et al (5), Bioenervi™ contains 67.6 µg/L of IGF-1. Therefore at a dose of 125 mL/day subjects would consume 8.45 µg of IGF-1 per day. Given that circulating concentrations of IGF-1 are on the order of 250 ng/mL in adult humans (2), and the average blood volume is 6 litres, the total quantity of IGF-1 in the circulation would be on the order of ~1,500 µg (excluding IGF-1 in other tissues). Therefore, if 100% of the IGF-1 in the Bioenervi™ supplement consumed in the study by Mero et al was absorbed, and accumulated only in the blood compartment (which is unlikely) then the total IGF-1 consumed during the study period (i.e. 8.45 µg/day x 8 days) would have been 67.6 µg, and would be expected to increase circulating IGF-1 concentrations by ~10 ng/ml (assuming no turnover). Mero reported an increase in circulating IGF-1 concentration of 5 nmol/L (i.e. 38.2 µg/L) which, in a volume of 6 litres, would require the addition of 229.2 µg of IGF-1 to the blood compartment. This quantity of IGF-1 represents 3.4 fold the total dose provided by the Bioenervi™ supplement consumed in the study by Mero et al (5), indicating that it is unlikely that the increase in IGF-1 reported in that study resulted from direct absorption of IGF-1 from the Bioenervi™ supplement, but would also have required an increase in endogenous IGF-1 production. In any case, regardless of the mechanism by which IGF-1 was increased in the study by Mero et al (5), the increase was not associated with any effect on physical performance.

9. The product used in the '037 application is currently sold as INTACT® INTACT® contains 1 – 2 mg/kg (~1.5mg/kg) of IGF-1. Therefore an oral dose of 60g of INTACT® per day will deliver approximately 0.09 mg IGF-1 per day. Clinical trials have shown improved physical performance after daily supplemention with INTACT® for 8 weeks at doses of 20g/day and 60g/day (1-3) but no change in circulating IGF-1 levels (1-4). Therefore current evidence suggests that while INTACT® can improve physical performance the effect is unlikely to be mediated by changes in circulating concentrations of IGF-1.

10. The references referred to above are as follows:

1) Buckley J, Abbott M, Brinkworth G, and Whyte P. Bovine colostrum supplementation during endurance running training improves recovery, but not performance. J Med Sci Sport 5: 65-79, 2002. **(Exhibit B)**

2) Buckley J, Brinkworth G, and Abbott M. Effect of bovine colostrum on anaerobic exercise performance and plasma insulin-like growth factor I. J Sports Sci 21: 577-588, 2003. **(Exhibit C)**

3) Coombes J, Conacher M, Austen S, and Marshall P. Dose effects of oral bovine colostrum supplementation on physical work capacity in cyclists. Med Sci Sport Exerc 34: 1184-1188, 2002 **(Exhibit D)**

4) Kuipers H, van Breda E, Verlaan G, and Smeets R. Effects of oral bovine colostrum supplementation on serum insulin-like growth factor I levels. Nutrition 18: 566-567, 2002 **(Exhibit E)**

5) Mero A, Miikkulainen H, Riski J, Pakkanen R, Aalto J, and Takala T. Effects of bovine colostrum supplementation on serum IGF-1, IgG, hormone, and saliva IgA during training. Journal of Applied Physiology 83: 1144-1151, 1997 **(Exhibit F)**

11. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: _____ Dated: 19 November 2006

2692.1

Bovine Colostrum Supplementation during Endurance Running Training Improves Recovery, but not Performance

JD Buckley[1], MJ Abbott[1], GD Brinkworth[1] & PBD Whyte[2]

[1]Centre for Research in Education and Sports Science, School of Physical Education Exercise and Sport Studies, University of South Australia, Adelaide, South Australia.
[2]Numico Research (Australia) Pty Ltd, Adelaide, South Australia.

Buckley, J.D., Abbott, M.J., Brinkworth, G.D., & Whyte, P.B.D. (2002). Bovine colostrum supplementation during endurance running training improves recovery, but not performance. *Journal of Science and Medicine in Sport* 5 (2): 65-79.

This study examined the effect of supplementation with concentrated bovine colostrum protein powder (Intact™) on plasma insulin-like growth factor I (IGF-I) concentrations, endurance running performance and recovery. Thirty physically active males completed 8 weeks of running training whilst consuming 60 g·day^{-1} of Intact™ powder (n=17) or a concentrated whey protein powder placebo (n=13) in a randomised, double-blind, parallel design. Plasma IGF-I concentrations were measured prior to subjects performing two (~30 min) incremental treadmill running tests to exhaustion (RUN1 and RUN2) separated by 20 min of passive recovery at Weeks 0, 4 and 8. Plasma IGF-I concentrations showed little change in either group (p=0.83). Effective peak running speed (PRS_E: i.e. equivalent of peak power) during RUN1 was not different between groups at Week 0 (p>0.99), and had increased by a similar amount in both groups by Week 4 (mean±SD, Intact™ 2.2±4.0%, placebo 3.2±3.3%; 95% confidence interval [95% CI] 15.7 to -13.7%; p=0.89) and Week 8 (Intact™ 3.6±5.6%, placebo 3.4±4.4 %; 95% CI -100.0 to 100.0 %; p>0.99). PRS_E was less in both groups during RUN2 (p<0.05), but was not significantly different between groups at Week 0 (p>0.99). PRS_E during RUN2 tended to have increased more in the placebo group by Week 4 (Intact™ 1.8±4.8%, placebo 4.2±3.9%; 95% CI 0.2 to -5 0%; p=0.07), but the Intact™ group had increased PRS_E significantly more by Week 8 (Intact™ 4.6±6.1%, placebo 2.0±4.5%; 95% CI 0.0 to 5.2%; p=0.05), resulting in a significantly faster PRS_E (p=0.003). We conclude that supplementation with Intact™ powder did not increase plasma IGF-I concentrations or improve performance during an initial bout of incremental running to exhaustion in our sample. However, performance during a second bout of exercise may be improved by as much as 5.2% in the average subject after 8 weeks of supplementation, possibly due to an enhancement of recovery.

Introduction

Bovine colostrum is the first milk secreted by cows after parturition and contains a number of bioactive proteins, including growth factors (Donovan & Odle, 1994; Francis et al., 1988; Schams, 1994), of which insulin-like growth factor I (IGF-I) is one of the most abundant and well-characterised (Francis et al., 1988).

Dietary colostrum has been shown to increase circulating IGF-I concentrations (Burrin et al., 1995; Ronge & Blum, 1988) and skeletal muscle protein synthesis (Burrin et al., 1992; Burrin et al., 1995) in newborn animals. More recently, supplementation with bovine colostrum has also been shown to

1.2692

increase serum IGF-I concentrations in adult humans (Mero et al., 1997). IGF-I stimulates lipoprotein lipase activity in adipocytes (Kern et al., 1985; Kern et al., 1989), and the administration of IGF-I in humans has been shown to increase lipolysis (Hussain et al., 1994) and lipid oxidation (Froesch et al., 1996). Circulating levels of IGF-I have also been shown to correlate with peak oxygen uptake ($\dot{V}O_{2peak}$) (Kelly et al., 1990; Poehlman & Copeland, 1990).

Endurance exercise training typically increases maximal or peak oxygen uptake ($\dot{V}O_{2peak}$) (Holloszy, 1973) and reduces blood lactate concentrations during submaximal exercise (Bergman et al., 1999; Hurley et al., 1984). Perhaps the most important adaptation in terms of attenuating the blood lactate response to exercise is an increase in the ability of trained muscle to oxidize fat (Hurley et al., 1986; Kiens, 1997; Kiens et al., 1993). Given the relationship between circulating IGF-I concentrations and $\dot{V}O_{2peak}$, as well as the ability of IGF-I to increase fat oxidation (Froesch et al., 1996), it was of interest to determine whether bovine colostrum supplementation could improve endurance exercise performance by enhancing the effects of training on $\dot{V}O_{2peak}$, increasing fat metabolism, and reducing blood lactate concentrations during exercise.

In addition to potential enhancements of endurance exercise performance, it has been proposed that colostrum supplementation might improve recovery from exercise (Anderson, 1994; Mero et al., 1997). The only study to date which has addressed the effect of colostrum supplementation on recovery found that a short period of supplementation (~5 days) had no effect on recovery from strength-speed training (Mero et al., 1997). However, the 5 day supplementation period may not have been long enough for any potential changes in recovery to be manifested. No studies have investigated the effects of bovine colostrum supplementation on recovery from endurance exercise.

The purpose of the present study was to determine whether a relatively long period of oral supplementation with bovine colostrum could increase plasma IGF-I concentrations, enhance endurance running performance and improve recovery.

Methods and procedures

Subjects

Subjects were 18 to 35 year old males (mean±SD, intact™ 25.2±4.2 yr [n=17], placebo 26.6±4.4 yr [n=13]; p=0.38) who had been participating in regular physical activity for at least three months prior to study commencement. All subjects were medically screened using a modified pre-exercise screening questionnaire prior to undertaking exercise testing (Olds & Norton, 1999). The protocol and the potential risks and benefits were fully explained to each subject before they provided written informed consent. All experimental procedures were approved by the Human Research Ethics Committee of the University of South Australia.

Experimental protocol

The study was carried out using a randomised, double-blind, placebo controlled, parallel design. All subjects were initially familiarised (2 wk) with the training, nutritional and testing procedures which would be carried out during the study.

three separate occasions at four weekly intervals for testing (Weeks 0, 4 and 8) after a minimum four hour fast. Tests were conducted at the same time of day to avoid circadian effects. At each testing session body mass and stature were measured prior to a venous blood sample being drawn from a forearm vein for determination of the plasma IGF-I concentration. Each subject then performed two incremental treadmill running tests (RUN1 and RUN2) to volitional exhaustion whilst expired air was collected for determination of gas exchange parameters, and fingertip blood samples were taken for determination of blood lactate concentrations. RUN1 and RUN2 were separated by a 20 min period of passive recovery during which subjects remained seated.

After being tested at Week 0, subjects were randomly allocated to consumption of 60 g•day^{-1} of either intact™ concentrated bovine colostrum protein powder containing 2 mg•kg^{-1} of IGF-I (Numico Research (Australia) Pty Ltd, Adelaide, Australia), or 60 g•day^{-1} of a concentrated whey protein powder placebo (Alacen, New Zealand Milk Products, Sydney, Australia). The concentration of IGF-I in the intact™ powder was determined using the same method, described below, as was used for determining the IGF-I concentration in the plasma samples. All supplements were provided in pre-packed 20g sachets and subjects consumed the contents of one sachet with their morning meal, and two sachets with their evening meal. The contents of each sachet were mixed with 85ml of warm water and 40ml of milk, shaken vigorously, and then chilled before drinking. The taste and color of the intact™ powder and the placebo were indistinguishable. On the day following the initial testing session subjects began taking the appropriate supplement and commenced a 3 day per week running training program. Subjects did not use any additional nutritional supplements during the study period, and food intakes were recorded daily for subsequent dietary analysis.

Body mass and stature

Body mass was measured using electronic digital scales (AND Mercury, FV-150, Tokyo, Japan). Stature was measured using a stadiometer (SECA, Hamburg, Germany) with subjects in the free-standing position (Norton & Olds, 1996).

Treadmill running tests

Baseline physiological data were collected whilst subjects stood quietly on the treadmill (Quinton Instruments, Model 1860, Washington, USA) for 3 min prior to commencing each running test. Subjects then commenced running at a speed of 10km•hr^{-1} and 0% grade. The treadmill speed remained constant throughout the test and the work load was incremented every 3 minutes by increasing the slope of the treadmill by 1% grade until the subject reached volitional exhaustion. Preliminary experiments indicated that subjects reach exhaustion after ~30 min using this protocol.

The treadmill % grade at exhaustion was used to predict the effective peak horizontal running speed (PRS_E) at exhaustion by combining two formulae from Brooks et al. (1996) to give:

$$PRS_E = S + 0.05*t*S$$

Where:

PRS_E = effective peak horizontal running speed ($km \cdot hr^{-1}$)
S = treadmill speed (i.e. 10 $km \cdot hr^{-1}$)
i = treadmill % grade at exhaustion

The technical error of measurement (TEM) for the PRS_E was 1%. This TEM was determined from two tests, separated by one week, carried out on five subjects who did not participate in the experiment. The first test was conducted 2 weeks after a familiarisation trial.

Cardiorespiratory variables

Measurements of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were recorded as 30 second averages throughout each treadmill run and the values averaged over the final 30 sec of each work load were recorded as the measured values. Subjects breathed through a low resistance respiratory valve (Hans Rudolph 2700 series, Kansas City, USA) with a pre-calibrated large flow turbine transducer (P.K. Morgan Mark 2, Seaford, Australia) attached to the inspiratory port to measure ventilatory volumes. Expired air was directed to a 2.6 L mixing chamber (Sportech, Canberra, Australia) from which dried gas was sampled continuously (~ 500 $ml \cdot min^{-1}$) and passed to an oxygen analyser (Ametek S-3A/I, Pittsburgh, USA) and a carbon dioxide analyser (Ametek CD-3A, Pittsburgh, USA), both of which had been calibrated prior to each exercise test with commercially-produced gas mixtures of known O_2 and CO_2 percentages (BOC Gases, Adelaide, Australia). The electrical outputs from the ventilation meter and gas analysers were integrated using a personal computer which calculated the necessary ventilatory variables. Heart rate (HR) was recorded as 5 second averages during the 3 min rest period prior to each treadmill run and during each run using a Sport Tester heart rate microcomputer and chest transmitter (Polar Accurex Plus, Polar Electro, Oulu, Finland). The 5 second averages at the end of the 3 min rest period prior to each treadmill run, at the end of each 3 min work load, and at the end of exercise were taken to be the measured values for HR.

Blood collection and analysis

For determination of the plasma IGF-I concentration, blood samples (4 ml) were drawn from an antecubital vein prior to performing any exercise at each testing session. The blood samples were placed into tubes containing 9 mg of di-potassium EDTA and centrifuged for 10 min at 2000g and 4°C using a refrigerated centrifuge (Beckman GS-6R, Palo Alto, USA). The plasma was then drawn off and stored in a polypropylene tube below -20°C for determination of plasma IGF-I concentrations at the end of the study (i.e. less than 3 months after sample collection).

The plasma concentration of IGF-I was measured in each sample three times by radioimmunoassay after separation of IGF binding proteins by high performance size exclusion liquid chromatography at pH 2.5 according to the method of Scott and Baxter (Scott & Baxter, 1986) as modified by Owens et. al. (Owens et al., 1994; Owens et al., 1990). Recombinant human IGF-I (hIGF-I) was obtained from GroPep Pty. Ltd. Adelaide, Australia. Radioligand was prepared to a specific activity of ~90 $Ci \cdot g^{-1}$ with chloramine-T and NaI^{125} (Amersham Pharmacia Biotech Inc, San Francisco, USA). Antiserum to hIGF-I was raised in rabbit. Samples were stripped of IGF-binding proteins in

fourteen chromatography sessions. The fraction containing IGF-I routinely eluted from the size exclusion HPLC column between 8.75 and 11.0 minutes after injection of the acidified plasma samples. The recovery from the column estimated from injections of [^{125}I]-iodo-IGF-I was 94±5% (mean±SD, n=13). All samples from each individual subject were included in the same chromatography session and measured in the same assay. The average minimal detectable concentration was 14 $ng \cdot ml^{-1}$ (range 12 to 19 $ng \cdot ml^{-1}$) and the average half-maximal response in the assay was produced by a sample containing 271 $ng \cdot ml^{-1}$ (range 235 to 315 $ng \cdot ml^{-1}$). For replicates of a quality control plasma specimen whose average IGF-I concentration was determined to be 70 $ng \cdot ml^{-1}$ after being measured three to five times in each assay, the average within and between assay TEMs were 10% and 16% respectively.

Blood lactate concentrations were determined from fingertip blood samples (50 µl) taken at the end of the 3 min rest period prior to each treadmill run, at the end of each 3 minute work load during the runs, and immediately upon the cessation of exercise. 25µl of each sample was immediately passed through an automated lactate analyser (Yellow Springs International, Model 1500 Sport, Yellow Springs, USA). The remainder of each sample was discarded.

Lactate threshold

A log-log transformation (Beaver et al., 1985) of the $\dot{V}O_2$ and the blood lactate concentrations during RUN1 at each testing session were used to determine the $\dot{V}O_2$ corresponding to the lactate threshold. Linear regression analysis of the HR vs $\dot{V}O_2$ response during exercise was then used to determine the HR corresponding to the $\dot{V}O_2$ at the lactate threshold. This HR was used as the training HR (HR_t) for the running training program.

Training program

Subjects were provided with a HR monitor (Polar Beat, Polar Electro, Finland) and ran for 45 min, 3 times per week at HR_t. During the first 4 weeks of the study they ran at the HR_t determined at week 0. During the second 4 weeks of the study they ran at the HR_t determined at week 4.

Nutrition

Subjects were provided with a copy of, and were instructed to eat according to, the 12345+ Food and Nutrition Plan (Jackson, 1991). Subjects were required to keep a daily food diary for the duration of the study and an analysis of dietary intakes was carried out using the SERVE Dietary Analysis Software program (M & H Williams Pty Ltd, Adelaide, Australia). Total energy intakes and percentages of energy intake contributed by carbohydrate, fat and protein were averaged over the first and second 4 weeks of the study to give mean daily intakes for each 4 week period.

Statistics

The student t-test was used to compare the ages of the groups. Univariate two-way analysis of variance (ANOVA) with repeated measures was used to determine the effect of the treatment, time of measurement, and their interactions on height and body mass. Relationships between variables were determined using linear regression analysis. To determine the effects of the treatment, time of measurement, and their interactions on the PRS_E, the log

of the PRS_E was taken to avoid non-uniform residuals, and these data were then analysed using univariate three-way ANOVA with repeated measures. One factor was the treatment group (i.e. intact™ powder or placebo) and the other factors (with repeated measures) were the treadmill run (i.e. RUN1 or RUN2) and the week of testing (i.e. Week 0, 4 or 8). Univariate two-way ANOVA (treatment x week) with repeated measures was used to determine the effect of the treatment and time of measurement on plasma IGF-1 concentrations. Univariate four-way ANOVA (treatment x run x week x time during run) with repeated measures was used to determine effects on other dependent variables (i.e. $\dot{V}O_2$, HR, blood lactate, RER) during exercise. ANOVA models incorporated a Greenhouse-Geisser correction for multisample asphericity. Where ANOVA showed a statistically significant main effect, pair-wise comparisons were performed using Tukey's test for Honestly Significant Differences. 95% confidence intervals (95% CI) are shown for the between-group differences in the within-group increases in PRS_E. A level of $p \leq 0.05$ was taken as indicating statistical significance. Unless otherwise stated, all values cited in the text and shown in figures represent means±SD.

Results

Height and body mass

There was little difference in height (intact™ 180.7±5.0 cm, placebo 177.1±5.5 cm; $p=0.09$), or body mass (intact™ 77.2±7.8kg, placebo 76.5±12.1kg; $p=0.90$) between the groups at Week 0, and neither height ($p=0.14$), nor body mass ($p=0.23$) had changed significantly in either group by Week 8.

Treadmill runs

There was no significant difference in PRS_E achieved during RUN1 between the two groups at Week 0 ($p>0.99$, Fig. 1). PRS_E during RUN1 increased in both groups during the study period, but there was still no difference between groups at Week 4 ($p>0.99$) or Week 8 ($p>0.99$). Expressed as percentage improvements, these increases in PRS_E were not different between groups at Week 4 (intact™) 2.2±4.0%, placebo 3.2±3.3%, 95% CI 15.7 to -13.7%; $p=0.89$), or at Week 8 (3.6±5.6%, placebo 3.4±4.4%, 95% CI -100 to 100%; $p>0.99$). The PRS_E for RUN2 was less than that achieved during RUN1 in both groups at all 3 testing sessions ($p<0.05$). There was no difference in PRS_E during RUN2 between the two groups at Week 0 ($p>0.99$, Fig. 1) or week 4 ($p=0.49$), but by week 8, PRS_E was significantly faster in the intact™ group compared with the placebo group ($p=0.003$). Expressed as percentage improvements, the increase in PRS_E was not different between groups at Week 4 (intact™ 1.8±4.8%, placebo 4.2±3.9%, 95% CI 0.2 to -5.0%; $p=0.07$), but was significantly greater in the intact™) group by Week 8 (intact™ 4.6±6.1%, placebo 2.0±4.5%, 95% CI 0.0 to 5.2%; $p=0.05$).

There were no significant differences in the $\dot{V}O_2$ response to submaximal exercise between the two groups during either of the runs at Weeks 0, 4, or 8 ($P>0.80$). The $\dot{V}O_{2peak}$ achieved at Week 0 (RUN1) was not significantly different between the two groups (intact™ 53.2±5.4 ml•kg^{-1}•min^{-1}, placebo 53.9±7.1 ml•kg^{-1}•min^{-1}; $p=0.91$) and $\dot{V}O_{2peak}$ changed little in either group during the study period ($p=0.58$). The $\dot{V}O_2$ (intact™ 37.6±8.3 ml•kg^{-1}•min^{-1}, placebo 40.2±5.4 ml•kg^{-1}•min^{-1}; $p=0.68$), and $\%\dot{V}O_{2peak}$ (intact™ 70.7±13.7%, placebo 75.0±7.8%; $p=0.58$) at the lactate threshold was not significantly different between the two groups at week 0, and neither the $\dot{V}O_2$ ($p>0.99$), nor the $\%\dot{V}O_{2peak}$ ($p=0.86$) at the lactate threshold changed significantly in either group during the study period.

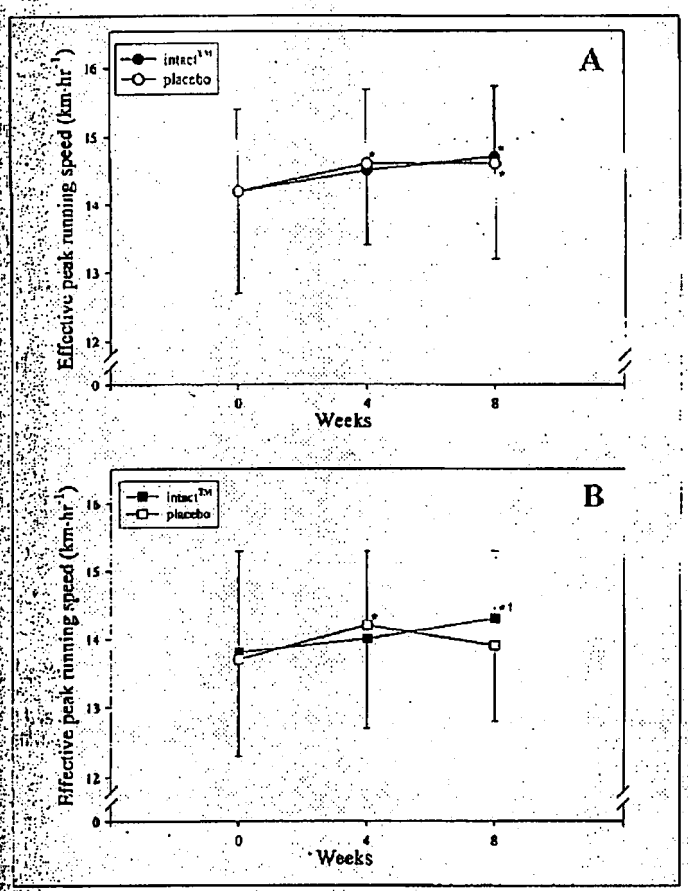

*Figure 1: Effective peak running speed (i.e. equivalent of peak power) during two incremental treadmill runs to exhaustion (RUN1 - panel A, RUN2 - panel B) separated by 20 min of recovery, prior to, and after 4 weeks and 8 weeks of endurance training and oral supplementation with intact™ concentrated bovine colostrum protein powder or concentrated whey protein powder (placebo). *p<0.01 compared with week 0. †p=0.003 compared with placebo.*

There was little difference in peak HR (HR_{peak}) between groups at Week 0 (intact™ 194±10 beats•min^{-1}, placebo 192±8 beats•min^{-1}; $p=0.26$). HR_{peak} decreased in both groups during the study period ($P<0.001$), with no significant difference in the magnitude of the decrease between groups ($p=0.47$), such that by Week 8 HR_{peak} was 190±8 beats•min^{-1} in the intact™ group and 187±8 beats•min^{-1} in the placebo group. HR at the lactate threshold (i.e. training heart rate; HR_t) was not significantly different between groups at Week 0 (intact™ 163±18 beats•min^{-1}, placebo 165±12 beats•min^{-1}; $p=0.60$).

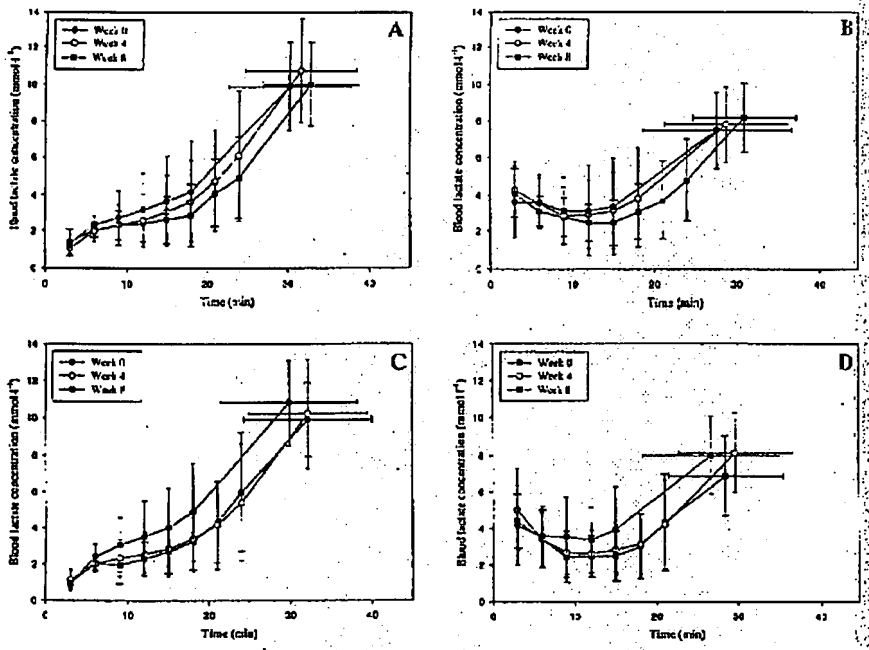

Figure 2: Blood lactate concentrations during an initial incremental treadmill run to exhaustion (intact™ - panel A, placebo - panel C) and a second incremental treadmill run to exhaustion after a 20 min recovery period (intact™ - panel B, placebo - panel D) prior to, and after 4 weeks and 8 weeks of endurance training and oral supplementation with intact™ concentrated bovine colostrum protein powder or concentrated whey protein powder (placebo).

HR_t decreased in both groups during the study period (p=0.04), reaching 157±14 beats•min^{-1} in the intact™ group and 156±10 beats•min^{-1} in the placebo group by Week 8, but the extent of the decrease was not significantly different between the groups (p=0.50).

Throughout the study period there was no significant difference between groups in the blood lactate response to submaximal exercise during either of the two runs (p=0.23, Fig. 2). There was an attenuation of the submaximal exercise blood lactate response during both runs as the study progressed (p=0.005), but the magnitude of the attenuation was similar in both groups (p=0.23). The blood lactate concentration decreased during the 20 min recovery period between treadmill runs in both groups at all three testing sessions (p<0.0001), but had not returned to pre-exercise concentrations in either group by the end of recovery at any of the three testing sessions (p<0.0001). The peak blood lactate concentrations reached by the end of RUN2 at each testing session were significantly lower than those reached at the end of RUN1 (p<0.001), but there was no significant difference between the two groups (p=0.28).

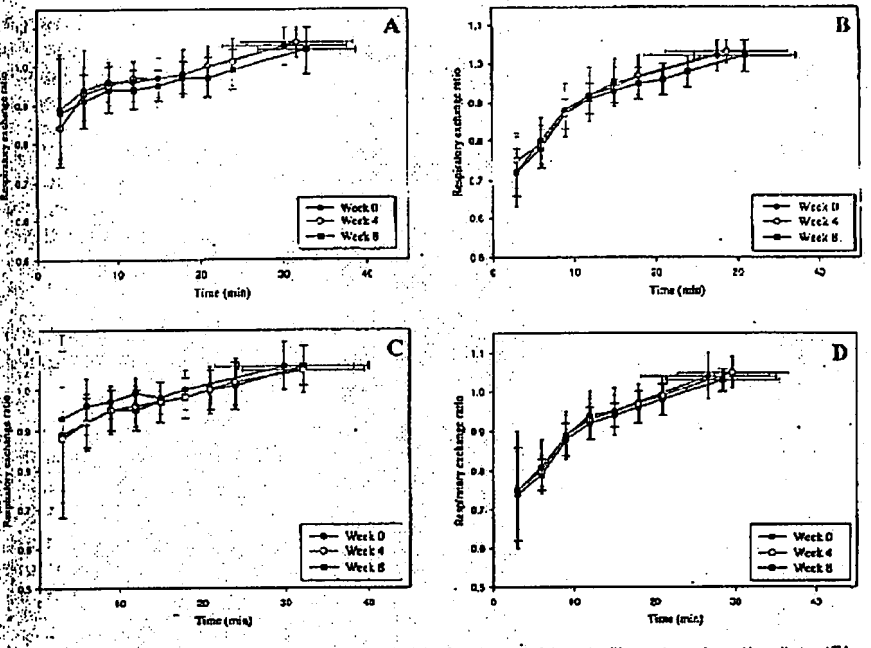

Figure 3: Respiratory exchange ratio during an initial incremental treadmill run to exhaustion (intact™ - panel A, placebo - panel C) and a second incremental treadmill run to exhaustion after a 20 min recovery period (intact™ - panel B, placebo - panel D) prior to, and after 4 weeks and 8 weeks of endurance training and oral supplementation with intact™ concentrated bovine colostrum protein powder or concentrated whey protein powder (placebo).

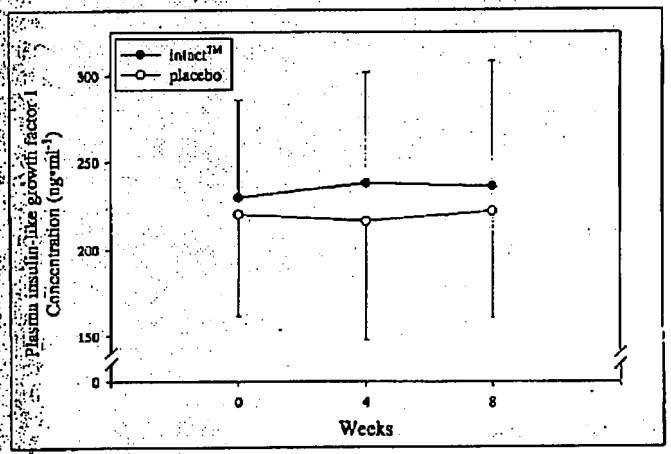

Figure 4: Plasma insulin-like growth factor 1 concentrations prior to, and after 4 and 8 weeks of endurance running training and oral supplementation with intact™ concentrated bovine colostrum protein powder or concentrated whey protein powder (placebo).

Throughout the study period there was no significant difference between groups in the RER response to submaximal exercise during either of the two runs (p=0.71, Fig. 3), and the RER response to submaximal exercise did not change significantly during the study period (p=0.20). The RER decreased in both groups at all three testing sessions during the 20 min recovery period between treadmill runs, reaching values lower than the RER prior to RUN1 (p<0.0001). The RER reached by the end of RUN2 at each testing session was significantly lower than the RER reached at the end of RUN1 (p<0.001), but there was no significant difference between the two groups (p=0.60) and these values changed little in either group during the study period (p=0.75).

Plasma insulin-like growth factor 1

Plasma IGF-1 concentrations were not significantly different between the two groups at week 0 (p=0.51, Fig. 4) and the plasma IGF-1 concentration changed little in either group over the 8 week study period (p=0.83).

Dietary intake

There was no significant difference in mean daily energy intake between the two groups during the first 4 weeks of the study (intact™ 9192±2501 kJ•day^{-1}, placebo 9204±1648 kJ•day^{-1}; p=0.49) and mean daily energy intake did not change significantly in either group during the final 4 weeks of the study period (p=0.42). Similarly, there were no significant differences in the percentages of mean daily dietary energy intake accounted for by carbohydrate (intact™ 46.5±5.2%, placebo 46.6±5.7%; p=0.80), protein (intact™ 24.2±2.9%, placebo 24.6±2.5%; p=0.98), or fat (intact™ 26.9±4.5%, placebo 26.0±5.7%; p=0.94) between the two groups during the first 4 weeks of the study, and these values did not change significantly in either group during the final 4 weeks of the study period (P>0.42).

Discussion

This study examined the effect of oral supplementation with bovine colostrum on endurance exercise performance and recovery. The principal finding was that supplementation with intact™ concentrated bovine colostrum protein powder during 8 weeks of training had little effect on performance during an initial bout of incremental running exercise to exhaustion, but did improve performance in a second bout of exercise after a 20 min recovery period.

It was hypothesised that any improvement in endurance exercise performance resulting from bovine colostrum supplementation would be mediated by an increase in the circulating IGF-I concentration. However, despite Mero et. al. (1997) previously demonstrating that bovine colostrum supplementation could increase the serum IGF-I concentration, supplementation with intact™ powder had little effect on the plasma IGF-I concentration in the present study. The increase in serum IGF-I reported by Mero et. al. (1997) occurred after only 8 days of supplementation, and it is possible therefore that the increase was only transient and was not detected in the present study because of the relatively long interval (i.e. 4 weeks) between the commencement of supplementation and the first assessment of changes in IGF-I. Alternatively, differences in the bioavailability of IGF-I between the supplements used in the two studies may have contributed to the different findings, but this would imply that the increase in serum IGF-I reported by

Mero et. al. (1997) was due to absorption of IGF-I from the colostrum supplement, and these authors were unable to determine whether the additional IGF-I was absorbed from the supplement, or resulted from an increase in endogenous IGF-I production. There is evidence from studies in neonatal animals that IGF-I in milk retains bioactivity within the gastrointestinal tract (Baumrucker et al., 1992; Philipps et al., 1995) and that orally administered ^{125}I-IGF-I can be transported into the circulation (Baumrucker et al., 1992; Donovan et al., 1997; Xu & Wang, 1996), but gut closure occurs during the first two days after birth (Westrom et al., 1984), and macromolecular transport ceases a short time after (Weaver & Walker, 1989). It is therefore difficult to see how a molecule such as IGF-I, with a molecular weight of 7.5 kDa (Rechler & Nissley, 1991), would be absorbed in significant quantities in the adult gastrointestinal tract. Further investigation is required to better characterise the time-course of any changes in circulating IGF-I, and to identify the source of any additional IGF-I, resulting from bovine colostrum supplementation.

An incremental running test to exhaustion, as used in the present study, is generically an incremental test to peak power (Hopkins et al., 1999). Perhaps the best outcome measure from such a test is peak power or its equivalent, because there is a direct linear relationship between peak power achieved in an incremental test and the mean power output in competitive endurance events (Hawley & Noakes, 1992). Distance covered or time to exhaustion in incremental exercise tests do not have this relationship because the tests do not start at zero power. With a treadmill test at fixed grade and increasing speed, the equivalent of peak power is peak speed. In the present study the speed was fixed and the grade was increased, which necessitated the grade at exhaustion to be converted to an equivalent horizontal speed (i.e. effective peak running speed, PRS_E) to provide an estimate of peak power (Brooks et al., 1996). The first treadmill run (i.e. RUN1) at each testing session provided a measure of endurance running performance and, although the training program provided a sufficient stimulus to induce improvements in PRS_E in both groups, the intact™ supplement did not provide any significant additional benefit. However, despite this apparent zero effect, the 95% CI's for the differences in improvement between the two groups were quite wide, indicating that the precision of the estimate of the effect of the supplement in this sample was quite poor. From this we can conclude that the intact™ supplement did not appear to have any effect on performance during RUN1 in the present subject sample, but the possibility of the supplement having a positive or negative effect on performance should not be excluded until a more precise estimate can be obtained through the testing of more subjects. The potential for bovine colostrum supplementation to enhance endurance running performance had been predicated on the ability of the supplement to increase the circulating IGF-I concentration, and thereby promote increases in $\dot{V}O_{2peak}$ and fat metabolism (assessed by measurements of RER). However, the supplement had no significant effect on plasma IGF-I concentrations in the present sample, so it is perhaps not too surprising that the supplement did not improve running performance.

Despite the intact™ supplement failing to improve performance during RUN1, there was good evidence that 8 weeks of supplementation provided a greater improvement in PRS_E during RUN2. Given that performances during

RUN1 were not significantly different between the two groups, it could be inferred that any differences in performance during RUN2 would reflect differences in the extent to which subjects were able to recover during the 20 min period between treadmill runs. Any such difference could not be attributed to differences in diet or training since, apart from the different supplements consumed, neither the dietary intakes, nor the training loads differed significantly between the two groups. There appeared to be little difference in recovery between the two groups prior to supplementation, because both groups achieved similar PRS_E during RUN2 at Week 0. Similarly, there was no significant difference in PRS_E between groups by Week 4. However, the 95% CI for the change in PRS_E by Week 4 overlapped zero, indicating that, although the intact™ supplement did not have a significant effect on PRS_E in our sample, until more subjects are tested we cannot exclude the possibility that 4 weeks of supplementation may have a small positive effect (0.2%) or a relatively large negative effect (-5%) on PRS_E during a second bout of incremental exercise. An improvement in PRS_E of only 0.2% over a 4 week period is unlikely to be of any great consequence, but athletes should take into account the possibility of a decrement of up to 5% during the first 4 weeks of supplementation when considering taking the intact™ supplement, at least until further studies are carried out. Irrespective of any potential negative effect during the first 4 weeks of supplementation, such an effect would appear to be only transient since, after 8 weeks of supplementation, the intact™ powder had provided a 2.6% greater improvement in PRS_E in our sample. Furthermore, unlike the values at Week 4, the 95% CI for the improvement in PRS_E by Week 8 did not overlap zero (0 to 5.2%), indicating that the likely range of the true effect of the intact™ supplement on PRS_E during a second bout of incremental exercise lies somewhere between no effect (i.e. 0%) and a 5.2% increase in PRS_E for the average subject. Although a positive effect of up to 5.2% would represent a substantial improvement for a training and supplementation period which was only 8 weeks in duration, it must not be forgotten that the subject cohort used in the present study consisted of active males, and it cannot necessarily be assumed that similar results would be obtained in well-trained or elite athletes, since such athletes almost certainly have a different training history and a physiological development that is closer to their genetic limits (Hopkins et al., 1999).

Although the apparent improvement in PRS_E during RUN2 tends to suggest that the intact™ supplement enhanced the ability to recover during the 20 min period between runs, the mechanism by which it exerted this effect could not be determined from the present data. The systemic physiological and metabolic parameters assessed (oxygen uptake, RER, blood lactate, heart rate) provided no evidence of a difference between the two groups during exercise, or by the end of the 20 min recovery period between runs. Perturbations within the tissues themselves, particularly the active skeletal muscles, may have recovered to a greater extent in the intact™ group without affecting any of these systemic parameters but, given that there are no published studies on the effects of colostrum supplementation on changes in muscle biochemistry during training, it is difficult to speculate about a potential mechanism. However, now that an effect has been demonstrated, further work should address the mechanism of action of this supplement.

Despite supplementation with intact™ powder not providing a direct benefit for endurance exercise performance per se, an improvement in recovery could indirectly facilitate performance improvements by allowing greater training impulses (frequency x duration x intensity) to be undertaken. It was not possible to test this hypothesis using the current data because the training impulses employed in the present study were controlled, such that both groups performed the same training whether they had the capacity to do more or not. It is also not clear whether the apparent improvement in recovery is limited to short-term recovery periods (i.e approx 20 min in duration), or whether there is some longer term benefit which extends over a period of hours or days. Further research is required to provide an answer to this question.

In conclusion, the present study demonstrated that 8 weeks of supplementation with 60 g•day^{-1} of intact™ powder had little effect on performance during an initial bout of incremental running exercise to exhaustion, but did improve performance in a subsequent exercise bout after a 20 min recovery period. The improved performance in the second exercise bout would seem to reflect an enhanced ability to recover from prior exercise, at least when the recovery period is 20 min in duration, but the mechanism underlying this benefit could not be identified. Further studies should use larger subject samples in order to gain a more precise estimate of the magnitude of the effect of the supplement, and should also investigate the supplement's mechanism(s) of action.

Acknowledgements

This work was supported jointly by funding from Numico Research (Australia) Pty Ltd, Adelaide, Australia and the Australian Industry Research and Development Board.

The authors gratefully acknowledge Andrew Modra and Simon Gunn for their assistance with some of the data collection for this study, and Dr Phillip Owens from the University of Adelaide, for performing the IGF-1 assays.

References

Anderson, O. (1994). Bioenervi™ floods Finland, but can it really cut recuperation times? **Running Research News 10**: 11.

Baumrucker, C. R., Hadsell, D. L., & Blum, J. W. (1992). Insulin-like growth factors (IGFs) and IGF binding proteins in mammary secretions: origins and implications in neonatal physiology. In: M. F. Picciano, & B. Linnerdal (Eds.), **Mechanisms Regulation Lactation and Infant Nutrients Utilization**. New York: Wiley-Liss, pp.285-308.

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1985). Improved detection of the lactate threshold during exercise using a log-log transformation. **Journal of Applied Physiology 59**: 1936-1940.

Bergman, B., Wolfel, E., Butterfield, G., Lopaschuk, G., Casazza, G., Horning, M., & Brooks, G. (1999). Active muscle and whole body lactate kinetics after endurance training in men. **Journal of Applied Physiology 87**: 1684-1696.

Brooks, G., Fahey, T., & White, T. (1996). **Exercise Physiology. Human Bioenergetics and its Applications** (2nd edn) Mountain View: Mayfield Publishing Company.

Burrin, D., Shulman, R., Reeds, P., Davis, T., & Gravitt, K. (1992). Porcine colostrum and milk stimulate visceral organ and skeletal muscle protein synthesis in newborn pigs. **Journal of Nutrition 122**: 1205-1213.

Burrin, E., Davis, T., Ebner, S., Schoknecht, P., Fiorotto, M., Reeds, P., & McAvoy, S. (1995). Nutrient-independent and nutrient-dependent factors stimulate proteins synthesis in colostrum-fed newborn pigs. **Pediatric Research 37**: 593-599.

Donovan, S., Chao, J. C.-J., Zijlstra, R., & Odle, J. (1997). Orally administered iodinated recombinant human insulin-like growth factor-I (^{125}I-rhIGF-1) is poorly absorbed by the newborn piglet. **Journal of Pediatric Gastroenterology and Nutrition 24**: 174-182.

Donovan, S. M., & Odle, J. (1994). Growth factors in milk as mediators of infant development. **Annual Review of Nutrition 14**: 147-167.

Francis, G. L., Upton, F. M., Ballard, F. J., McNeill, K. A., & Wallace, J. C. (1988). Insulin-like growth factors 1 and 2 in bovine colostrum. Sequences and biological activities compared with those of a potent truncated form. **Biochemical Journal 251**: 95-103.

Froesch, E., Hussain, M., Schmid, C., & Zapf, J. (1996). Insulin like growth factor 1: physiology, metabolic effects and clinical uses. **Diabetes Metabolism Review 12**: 195-215.

Hawley, J., & Noakes, T. (1992). Peak power output predicts maximal oxygen uptake and performance time in trained cyclists. **European Journal of Applied Physiology 65**: 79-83.

Holloszy, J. O. (1973). Biochemical adaptations to exercise: aerobic metabolism. **Exercise and Sport Sciences Reviews 1**: 45-71.

Hopkins, W., Hawley, J., & Burke, L. (1999). Design and analysis of research on sport performance enhancement. **Medicine and Science in Sports and Exercise 31**: 472-485.

Hurley, B. F., Hagberg, J. M., Allen, W. K., Seals, D. R., Young, J. C., Cuddihee, R. W., & Holloszy, J. O. (1984). Effect of training on blood lactate levels during submaximal exercise. **Journal of Applied Physiology 56**: 1260-1264.

Hurley, B. F., Nemeth, P. M., Martin III, W. H., Hagberg, J. M., Dalsky, G. P., & Holloszy, J. O. (1986). Muscle triglyceride utilization during exercise: effect of training. **Journal of Applied Physiology 60**: 562-567.

Hussain, M., Schmitz, O., Reisen, W., Steinmann, B., & Froesch, E. (1994). Five day treatment with IGF-1 alters circulating lipid and amino acid profiles in men. **Growth Regulation 4**: 1152.

Jackson, M. (1991). **The 12345+ Food and Nutrition Plan**. Adelaide: Anti Cancer Foundation of South Australia.

Kelly, P., Eisman, J., Stuart, M., Pocock, N., Sambrook, P., & Gwinn, T. (1990). Somatomedin-c, physical fitness, and bone density. **Journal of Clinical Endocrinology and Metabolism 70**: 718-723.

Kern, P., Graves, D., Baskin, J., Eckel, R., & van Wyk, J. (1985). Somatomedin C may be a local regulator of lipoprotein lipase in human adipose tissue. **Clinical Research 33**: 434A.

Kern, P. A., Svoboda, M. E., Eckel, R. H., & Van Wyk, J. J. (1989). Insulinlike growth factor action and production in adipocytes and endothelial cells from human adipose tissue. **Diabetes 38**: 710-717.

Kiens, B. (1997). Effect of endurance training on fatty acid metabolism: local adaptations. **Medicine and Science in Sports and Exercise 29**: 640-645.

Kiens, B., Essen-Gustavsson, B., Christensen, N. J., & Saltin, B. (1993). Skeletal muscle substrate utilization during submaximal exercise in man: Effect of endurance training. **Journal of Physiology 469**: 459-478.

Mero, A., Miikkulainen, H., Riski, J., Pakkanen, R., Aalto, J., & Takala, T. (1997). Effects of bovine colostrum supplementation on serum IGF-1, IgG, hormone, and saliva IgA during training. **Journal of Applied Physiology 83**: 1144-1151.

Norton, K., & Olds, T. (1996). **Anthropometrica**. Sydney: University of New South Wales Press.

Olds, T., & Norton, K. (1999). **Pre-exercise Health Screening Guide**. Champaign: Human Kinetics.

Owens, J. A., Kind, K. L., Carbone, F., Robinson, J. S., & Owens, P. C. (1994). Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. **Journal of Endocrinology 140**: 5-13.

Owens, P. C., Johnson, R. J., Campbell, R. G., & Ballard, F. J. (1990). Growth hormone increases insulin-like growth factor-I (IGF-I) and decreases IGF-II in plasma of growing pigs. **Journal of Endocrinology 124**: 269-275.

Philipps, A., Rao, R., Anderson, G., McCracken, D., Lake, M., & Koldovsky, O. (1995). Fate of insulin-like growth factors I and II administered orally to suckling rats. **Pediatric Research 37**: 586-592.

Poehlman, E., & Copeland, K. (1990). Influence of physical activity on insulin-like growth factor-I in healthy younger and older men. **Journal of Clinical Endocrinology and Metabolism 71**: 1468-1473.

Rechler, M., & Nissley, S. (1991). Insulin-like growth factors. In: M. Sporn, & A. Roberts (Eds.), **Peptide growth factors and their receptors I**. New York: Springer-Verlag, pp.263-368.

Ronge, H., & Blum, J. (1988). Somatomedic C and other hormones in dairy cows around parturition, in newborn calves and in milk. **Journal of Animal Physiology and Animal Nutrition 60**: 168-176.

Schams, D. (1994). Growth factors in milk. **Endocrine Regulation 28**: 3-8.

Scott, C. D., & Baxter, R. C. (1986). Production of insulin-like growth factor I and its binding protein in rat hepatocytes cultured from diabetic and insulin-treated diabetic rats. **Endocrinology 119**: 2346-2352.

Weaver, L., & Walker, W. (1989). Uptake of Macromolecules in the Neonate. In: E. Lebenthal (Ed.), **Human Gastrointestinal Development**. New York: Raven Press, pp.731-748.

Westrom, B., Svendsen, J., Ohlsson, B., Tagesson, C., & Karlson, B. (1984). Intestinal transmission of macromolecules (BSA and FITC-labelled dextran) in the neonatal pig. **Biology of the Neonate 46**: 20-26.

Xu, R., & Wang, T. (1996). Gastrointestinal absorption of insulinlike growth factor-I in neonatal pigs. **Journal of Pediatric Gastroenterology and nutrition 23**: 430-437.

Bovine Colostrum Supplementation during Endurance...

Journal of Sports Sciences, 2003, 21, 577–588

Taylor&Francis
healthsciences

Effect of bovine colostrum on anaerobic exercise performance and plasma insulin-like growth factor I

JONATHAN D. BUCKLEY,* GRANT D. BRINKWORTH and MARION J. ABBOTT

School of Health Sciences, University of South Australia, Holbrooks Road, Underdale, SA 5032, Australia

Accepted 21 March 2003

In this study, we examined the effects of bovine colostrum on peak vertical jump power (VJ_{peak}), peak cycle power (CP_{peak}), alactic anaerobic work capacity, resistance exercise one-repetition maxima (1-RM) and plasma insulin-like growth factor I (IGF-I) concentrations. Using a randomized, double-blind, placebo-controlled, parallel design, 51 males completed 8 weeks of resistance and plyometric training while consuming 60 g·day^{-1} of bovine colostrum ($n=26$) or concentrated whey protein powder ($n=25$). Peak vertical jump power, peak cycle power, alactic anaerobic work capacity, 1-RM and plasma IGF-I were not different between groups at baseline ($P>0.33$). Peak vertical jump power and peak cycle power were still not significantly different between groups by week 4 (VJ_{peak}: bovine colostrum, 7231±488 W; whey protein, 7214±530 W; $P=0.99$; CP_{peak}: bovine colostrum, 1272±202 W; whey protein, 1232±208 W; $P=0.99$). By week 8, however, peak vertical jump power (bovine colostrum, 7370±503 W; whey powder, 7237±481 W; 95% confidence intervals, 54 to 170 W; $P<0.01$) and peak cycle power (bovine colostrum, 1400±215 W; whey protein, 1311±192 W; 95% confidence intervals, 20 to 61 W; $P<0.01$) were significantly higher in the bovine colostrum condition. Alactic anaerobic work capacity and 1-RM increased ($P<0.001$), but the increases were not different between groups ($P>0.08$). Plasma IGF-I did not change in either group ($P=0.55$). We conclude that bovine colostrum supplementation during training significantly increased peak anaerobic power, but had no effect on alactic anaerobic work capacity, 1-RM or plasma IGF-I.

Keywords: alactic anaerobic capacity, alactic anaerobic power, one-repetition maximum, plyometric training, resistance training, vertical jump.

Introduction

Colostrum is the first milk secreted by mammals after parturition and contains high concentrations of bioactive components that are important for the development of the neonate (Pakkanen and Aalto, 1997). Feeding colostrum to neonatal animals has been shown to increase skeletal muscle protein synthesis (Burrin *et al.*, 1992, 1995), including the synthesis of contractile protein (Fiorotto *et al.*, 2000), leading to interest in the potential for colostrum supplementation to improve physical performance in humans.

In the first study of its kind, Mero *et al.* (1997) found that 8 days of supplementation with a low dose of a liquid form of bovine colostrum during speed and strength training had no effect on vertical jump height or recovery from exercise. Antonio *et al.* (2001) subsequently showed that a longer period of supplementation (i.e. 8 weeks) with a moderate dose (20 g·day^{-1}) of a powdered form of bovine colostrum during combined endurance and resistance training significantly increased lean body mass, but had no effect on the one-repetition maximum (1-RM) for bench press or on the performance of submaximal bench press exercise to exhaustion. More recently, Hofman *et al.* (2002) reported that 8 weeks of supplementation with a higher dose (60 g·day^{-1}) of concentrated bovine colostrum protein powder significantly improved repeat sprint ability and tended to improve vertical jump height compared with a whey protein placebo, and attributed the lack of a significant effect on vertical jump height to an insufficient sample size. One aim of the present study, therefore, was to re-examine the effect of a high dose (60 g·day^{-1}) of bovine colostrum powder on vertical jump performance using a larger sample size. Also, because the test of repeat sprint ability used by Hofman *et al.* (2002) only provided an estimate of average alactic anaerobic power, we sought to better characterize the effects of bovine colostrum supple-

* Author to whom all correspondence should be addressed:
e-mail: j.buckley@unisa.edu.au

Journal of Sports Sciences ISSN 0264-0414 print/ISSN 1466-447X online © 2003 Taylor & Francis Ltd
DOI: 10.1080/0264041031000101935

mentation on anaerobic exercise performance by assessing both peak anaerobic power and alactic anaerobic work capacity.

Despite finding no effect on anaerobic exercise performance or recovery, Mero *et al.* (1997, 2002) have shown that supplementation with bovine colostrum, using doses which delivered between 1.7 and 74 μg of IGF-I per day, increased circulating IGF-I concentrations in a dose-dependent manner. However, three subsequent studies from independent laboratories (Buckley *et al.*, 2002; Coombes *et al.*, 2002; Kuipers *et al.*, 2002) have reported no effect of bovine colostrum supplementation on circulating IGF-I concentrations, despite all three of these studies providing a dose of bovine colostrum that contained much more IGF-I (i.e. a dose of 120 μg of IGF-I per day) than in the studies by Mero *et al.* (1997, 2002). Given the potential for increased circulating IGF-I concentrations to improve muscular strength (Borst *et al.*, 2001), and that the effects of bovine colostrum supplementation on circulating IGF-I concentrations are equivocal, a secondary aim of the present study was to re-investigate the effect of bovine colostrum supplementation on circulating IGF-I concentrations.

Methods

Participants

The participants were males aged 18–35 years (mean ± *s*: bovine colostrum group, 23.5 ± 4.1 years, $n = 26$; whey protein group, 25.1 ± 5.1 years, $n = 25$; $P = 0.22$), who had been taking part in regular physical activity for at least 3 months before the study began. They all completed a modified health screening questionnaire before participation (Olds and Norton, 1999). The study protocol and the potential risks and benefits of the study were explained to each participant before they provided written informed consent. All experimental procedures were approved by the Human Research Ethics Committee of the University of South Australia.

Experimental design

The study used a randomized, double-blind, placebo-controlled, parallel design. All participants were familiarized with the training, nutritional and test procedures that were to be followed in the 2 weeks before the study commenced. The study lasted 8 weeks and each participant attended the laboratory on three separate occasions at intervals of 4 weeks (weeks 0, 4 and 8). The participants fasted for 4–12 h before each test session, depending on the time of day they were tested. Participants tested in the morning completed an overnight fast (i.e. 12 h), whereas those tested in the afternoon and evening completed a 4 h fast. Each participant was tested at the same time of day at each test session and underwent the same fasting protocol before each session.

At each test session, body mass and height were recorded before a venous blood sample was drawn from a forearm vein for determination of plasma IGF-I concentration. The participants then performed a warm-up consisting of jogging and stretching, followed by three short (~20 m) maximal sprints. Five minutes after the warm-up, each participant performed three maximal 10 s efforts on a cycle ergometer, each separated by 2 min of recovery, to determine peak anaerobic cycle power and alactic anaerobic work capacity. The peak anaerobic cycle power and alactic anaerobic work capacity from the best of the three efforts were recorded for analysis. The cycle test was followed by 5 min of recovery, after which the participants performed three maximal vertical jumps, each separated by 2 min of recovery. The best of the three jumps was recorded for analysis. After performing the vertical jump tests, each participant had their 1-RM assessed for several resistance training exercises (see Table 1). The 1-RMs determined at weeks 0 and 4 were used to calculate training loads for the subsequent 4 week training periods. On the day after the initial test session at week 0, the participants began taking the appropriate supplement and commenced a 6 day per week resistance and plyometric training programme. The participants were not permitted to use any additional nutritional supplements during the study period, and food intakes were recorded daily for subsequent dietary analysis.

Supplements

After being tested at week 0, the participants were randomly allocated to the consumption of 60 $g \cdot day^{-1}$ of either a concentrated bovine colostrum protein powder (intact™, Numico Research Australia Pty Ltd, Adelaide, Australia) or a concentrated whey protein powder placebo (Alacen, New Zealand Milk Products, Sydney, Australia). Both supplements were commercially available products and the nutritional composition of each of the supplements is provided in Table 2. All supplements were provided in pre-packed 20 g sachets and the participants consumed the contents of one sachet with their morning meal and the contents of two sachets with their evening meal. The contents of each sachet were mixed with 85 ml of warm water and 40 ml of milk, shaken vigorously, and then chilled before drinking. The taste and colour of the two supplements were indistinguishable.

Table 1. One-repetition maxima during 8 weeks of supplementation with concentrated bovine colostrum protein powder (intact™) or concentrated whey protein powder during combined resistance and plyometric training (mean ± *s*)

		Week 0	Week 4	Week 8
Bench press (kg)	BC	69.7 ± 16.5	76.1 ± 15.7	79.6 ± 15.1
	WP	73.4 ± 17.0	79.9 ± 17.2	81.9 ± 16.1
Chin-up (kg)	BC	95.7 ± 12.9	103 ± 14.7	107 ± 15.9
	WP	99.2 ± 13.9	103 ± 16.9	107 ± 16.3
Parallel dip (kg)	BC	104 ± 19.9	111 ± 20.6	119 ± 20.0
	WP	108 ± 21.2	116 ± 18.5	121 ± 19.1
Biceps curls (kg)	BC	41.3 ± 10.0	45.7 ± 8.2	47.3 ± 9.2
	WP	44.8 ± 8.2	47.8 ± 8.9	49.3 ± 8.9
Leg press (kg)	BC	213 ± 47.7	250 ± 55.9	278 ± 68.6
	WP	227 ± 72.8	262 ± 71.0	294 ± 77.1
Knee extension (kg)	BC	72.8 ± 23.4	85.5 ± 26.9	89.5 ± 23.8
	WP	66.9 ± 25.9	82.5 ± 25.0	87.5 ± 25.1
Knee flexion (kg)	BC	44.8 ± 12.2	50.2 ± 11.6	54.0 ± 12.1
	WP	46.4 ± 11.0	49.6 ± 11.8	53.5 ± 11.5
Calf raise (kg)	BC	222 ± 48.0	253 ± 44.0	265 ± 49.9
	WP	215 ± 48.9	242 ± 45.8	245 ± 56.6

Abbreviations: BC = concentrated bovine colostrum protein powder (intact™); WP = concentrated whey protein powder.

Table 2. Nutritional composition of a 60 g dose of concentrated bovine colostrum protein powder (intact™) and concentrated whey protein powder (Alacen)*

	Bovine colostrum	Whey protein
Energy (kJ)	1200	1020
Protein (g)	45	48
Fat (g)	2.1	3.9
Carbohydrate (g)	6.6	3.7
Calcium (mg)	720	210
Sodium (mg)	105	90
Potassium (mg)	300	300
IGF-I (μg)	120	Negligible

*Values reported by supplement manufacturers.

Body mass and height

Body mass was measured using electronic digital scales (A&D Mercury, FV-150, California, USA). Height was measured using a stadiometer (SECA, Hamburg, Germany) with the participant in the free-standing position (Norton and Olds, 1996).

Blood collection and analysis

Blood samples (4 ml) were drawn from an antecubital vein using standard aseptic techniques and placed into tubes containing 9 mg of di-potassium EDTA. The samples were centrifuged for 10 min at 2000 *g* and 4°C using a refrigerated centrifuge (Beckman GS-6R, Palo Alto, CA, USA) and the plasma was drawn off and stored in polypropylene tubes below −20°C for the determination of plasma IGF-I concentrations at the end of the study (i.e. less than 3 months after sample collection). The plasma concentration of IGF-I was measured in triplicate in a total of four assays by radioimmunoassay after separation of IGF-binding proteins by high-performance size exclusion liquid chromatography (HPLC) at pH 2.5 according to the method of Scott and Baxter (1986) as modified by Owens *et al.* (1990, 1994). Recombinant human IGF-I (*h*IGF-I) was obtained from GroPep Pty Ltd Adelaide. Radioligand was prepared to a specific activity of ~90 $Ci \cdot g^{-1}$ with chloramine-T and NaI^{125} (Amersham Pharmacia Biotech Inc., San Francisco, CA, USA). Antiserum to *h*IGF-I was raised in rabbits. Samples were stripped of IGF-binding proteins in 14 chromatography sessions. The fraction containing IGF-I was routinely eluted from the

size exclusion HPLC column 8.75–11.0 min after injection of the acidified plasma samples. The recovery from the column estimated from injections of [^{125}I]-iodo-IGF-I was 94 ± 5% (mean ± s; n = 13). All samples from each individual participant were included in the same chromatography session and measured in the same assay. The average minimal detectable concentration was 14 ng · ml^{-1} (range 12–19 ng · ml^{-1}) and the average half-maximal response in the assay was produced by a sample containing 271 ng · ml^{-1} (range 235–315 ng · ml^{-1}). For replicates of a quality control plasma specimen whose average IGF-I concentration was determined to be 70 ng · ml^{-1} after being measured 3–5 times in each of the four assays, the average within- and between-assay coefficients of variation were both 7%.

Peak anaerobic cycle power and anaerobic work capacity

Peak anaerobic cycle power and anaerobic work capacity were assessed during three attempts at a 10 s maximal exercise test (Telford *et al.*, 1989; Ellis *et al.*, 2000) using a front-access cycle ergometer (Repco Exertech, Melbourne, Australia) connected to a work monitor unit (Repco Exertech, Melbourne, Australia). The participants stood up on the cycle pedals with their preferred foot forward. A countdown from 3 was given before the participants performed maximal cycle exercise for 10 s. The participants were allowed a 2 min recovery between each of the three attempts, during which time they continued to rotate the cycle pedals to maintain venous return from the legs. The highest value for peak anaerobic cycle power and anaerobic work capacity from the three attempts was used for analysis. The typical error of measurement for this method of determining peak anaerobic cycle power and anaerobic work capacity in 10 male volunteers not participating in the present study, and who were tested on each of two consecutive days, was 2.4% for both measures.

Vertical jump power

The assessment of peak vertical jump power required the participants to jump and make a mark with their fingertips on a chalk board. A chalk board (1.0 m high × 0.25 m wide) was fixed to a wall and shaded with chalk dust. The participants' take-off height was measured by wetting the fingers of the preferred hand and recording the height of the uppermost mark made on the chalk board by the fingertips as the participants reached as far up the board as possible while standing on their toes. They then performed a counter-movement jump and endeavoured to touch the chalk board at the highest point possible with their wet fingers. As the fingertips contacted the board they left a clearly visible trail, of which the highest point was considered to be the maximum jump height. The actual height jumped was recorded as the difference between the take-off height and the maximum jump height as measured with a metal tape (Lufkin, W606PM, Raleigh, NC, USA). This procedure was repeated three times at each test session with a 2 min recovery period between jumps, with the best of the three jumps being recorded for analysis. Peak power developed during the vertical jump (VJ_p) was then estimated from the vertical jump height using the equation of Harman *et al.* (1991):

$$VJ_p\ (W) = (61.9 \times VJH) + (36.0 \times BM) + 1822$$

where VJH = vertical jump height (cm) and BM = body mass (kg). The typical error of measurement for this method of determining peak vertical jump power in 10 male volunteers not participating in the present study, and who were tested on each of two consecutive days, was 1.2%.

One-repetition maxima and training programme

The one-repetition maxima (1-RMs) for several resistance exercises (see Table 1) were assessed after measuring peak vertical jump power at weeks 0, 4 and 8. The values at weeks 0 and 4 were used to calculate the training loads for a resistance training programme, which was performed during each subsequent 4 week training period. Weight discs and/or dumb-bells were hung around the participant's waist, as appropriate, to determine the 1-RM for exercises that involved lifting one's own body mass (i.e. chin-ups and parallel dips). The resistance training programme was performed three times per week on alternate days to a plyometric training programme, which was also performed three times per week. The participants were allowed one rest day each week, which, at weeks 4 and 8, was the day on which the test protocol was conducted.

The loads used in the resistance training programme alternated between 90% and 35% 1-RM at successive training sessions, and three sets of each exercise were performed. When the 90% 1-RM load was used, the three sets were performed to failure. When the 35% 1-RM load was used, the participants performed three sets of 20 repetitions or exercised to failure, whichever occurred first. The order in which the resistance exercises in Table 1 are listed is the same order in which they were performed at each training session. The participants also performed non-resisted exercises consisting of sit-ups, hip flexions and twisting sit-ups. The sit-ups and twisting sit-ups were performed on an incline sufficient to cause failure in the third set at the 12th and 20th repetitions when training using the 90% and 35% 1-RM loads, respectively. Hip flexions were

only performed using the resistance offered by the weight of the participant's legs. Each training session was preceded by, and followed by, 5 min of light jogging and personal stretching. Rest periods between sets were not timed. Each participant kept a record of the number of repetitions performed during each set in a training diary to monitor compliance and to allow for subsequent calculation of the training volume.

The plyometric exercises and the durations of recovery allowed between repetitions are listed in Table 3. Each session of plyometric training was preceded by a warm-up consisting of an 800 m jog, personal stretching and 100 m run throughs at ~70%, ~80% and ~90% of maximum pace. All plyometric exercises were performed with maximal effort.

Table 3. Plyometric training exercises and recovery times during 8 weeks of supplementation with concentrated bovine colostrum protein powder (intact^{TM}) or concentrated whey protein during combined resistance and plyometric training

Exercise	Recovery time
2 × 20 m hopping on each leg	Untimed
2 × 50 m bounding	Untimed
10 × vertical jumps	Untimed
3 × 20 m sprints	1 min
3 × 50 m sprints	2 min
3 × 20 m sprints	1 min
1 × 200 m sprint	2 min
1 × 400 m sprint	End of session

Diet

The participants were provided with a copy of, and were instructed to eat according to, a healthy eating plan based on a diet pyramid (Jackson, 1991). They were required to keep a daily food diary for the duration of the study and an analysis of dietary intakes was carried out using the SERVE® Dietary Analysis Software program (M&H Williams Pty Ltd, Adelaide, Australia). Total energy intakes and percentages of energy intake contributed by carbohydrate, fat, protein and alcohol were averaged over the first and second 4 weeks of the study to give mean daily intakes for each 4 week period (Table 4).

Statistical analysis

Student's *t*-tests were used to compare differences between group means for age and for values at week 0. To determine the effects of the treatment, time of measurement and their interactions on the dependent measures, analysis of variance (ANOVA) with repeated measures (repeated measurement was week of testing, i.e. week 0, week 4 or week 8) was used, incorporating a Greenhouse-Geisser adjustment for sphericity. Where a significant main effect was found, pairwise comparisons were performed using Tukey's HSD test. The data for peak vertical jump power and peak cycle power were

Table 4. Dietary intakes during 8 weeks of supplementation with concentrated bovine colostrum protein powder (intact^{TM}) or concentrated whey protein powder during combined resistance and plyometric training (mean ± *s*)

		Weeks 0–4	Weeks 4–8
Mean daily energy intake (kJ · day^{-1})	BC	10516 ± 2198	10284 ± 2238
	WP	10539 ± 2377	10457 ± 2158
Carbohydrate as % daily energy intake	BC	45.8 ± 5.6	44.0 ± 5.9*
	WP	44.6 ± 4.3	42.9 ± 6.4*
Protein as % daily energy intake	BC	25.4 ± 3.8	25.7 ± 4.1
	WP	25.5 ± 3.3	26.3 ± 3.5
Mean daily protein intake per unit body mass (g · kg body mass^{-1})	BC	1.9 ± 0.4	1.8 ± 0.4
	WP	1.9 ± 0.4	1.9 ± 0.5
Fat as % daily energy intake	BC	27.9 ± 4.7	28.8 ± 5.2
	WP	29.3 ± 4.8	29.7 ± 5.1
Alcohol as % daily energy intake	BC	0.9 ± 1.5	1.5 ± 2.0†
	WP	0.6 ± 0.9	1.1 ± 1.9†

Abbreviations: BC = concentrated bovine colostrum protein powder (intact^{TM}); WP = concentrated whey protein powder.
*Significantly different from weeks 0–4 ($P = 0.05$). †Significantly different from weeks 0–4 ($P = 0.03$).

analysed in the same ANOVA by including the two tests as an additional repeated measurement (i.e. each participant performed both tests) to facilitate a direct comparison of the effects of the treatment on these two different measures of peak anaerobic power. Statistical significance was set at $P < 0.05$. The 95% confidence intervals (95% CI) are given for the between-group differences in the within-group changes in parameters where appropriate. The centrality and spread of values of variables are shown throughout as the mean ± standard deviation (*s*).

Results

Body mass and height

There was no significant difference in height (bovine colostrum, 1.79 ± 0.07 m; whey protein, 1.78 ± 0.07 m; $P = 0.59$) or body mass (bovine colostrum, 77.0 ± 9.7 kg; whey protein, 79.4 ± 9.0 kg; $P = 0.36$) between the two groups at week 0. Height did not change significantly in either group during the study period ($P = 0.44$). Body mass increased in both groups during the study ($P < 0.0001$), but by similar amounts such that there were still no differences at week 4 (bovine colostrum, 77.7 ± 9.6 kg; whey protein, 80.0 ± 8.8 kg; $P = 0.39$) or at week 8 (bovine colostrum, 77.8 ± 9.6 kg; whey protein, 80.0 ± 8.7 kg; $P = 0.38$).

Plasma insulin-like growth factor I concentration

Plasma IGF-I concentrations are shown in Fig. 1. There was no significant difference in plasma IGF-I concentration between the two groups at week 0 ($P = 0.87$) and the plasma concentration did not change significantly in either group during the study period ($P = 0.55$).

Peak anaerobic cycle power and anaerobic work capacity

There was no difference in peak cycle power between the two groups at week 0 ($P = 0.99$; Fig. 2). Peak cycle power did increase by a similar amount in both groups by week 4 ($P = 0.99$; 95% CI = −13,468 to 13,451 W), but had increased significantly more in the bovine colostrum group by week 8 ($P < 0.001$; 95% CI = 20 to 61 W), such that cycle peak power was significantly higher in the bovine colostrum group by the end of the study period ($P < 0.01$).

Anaerobic work capacity was not different between groups at week 0 (bovine colostrum, 9.5 ± 1.3 kJ; whey protein, 9.1 ± 1.5 kJ; $P = 0.34$). Anaerobic work capacity increased in both groups during the study period ($P < 0.001$), but the increases were not different between groups ($P = 0.23$), such that there were no

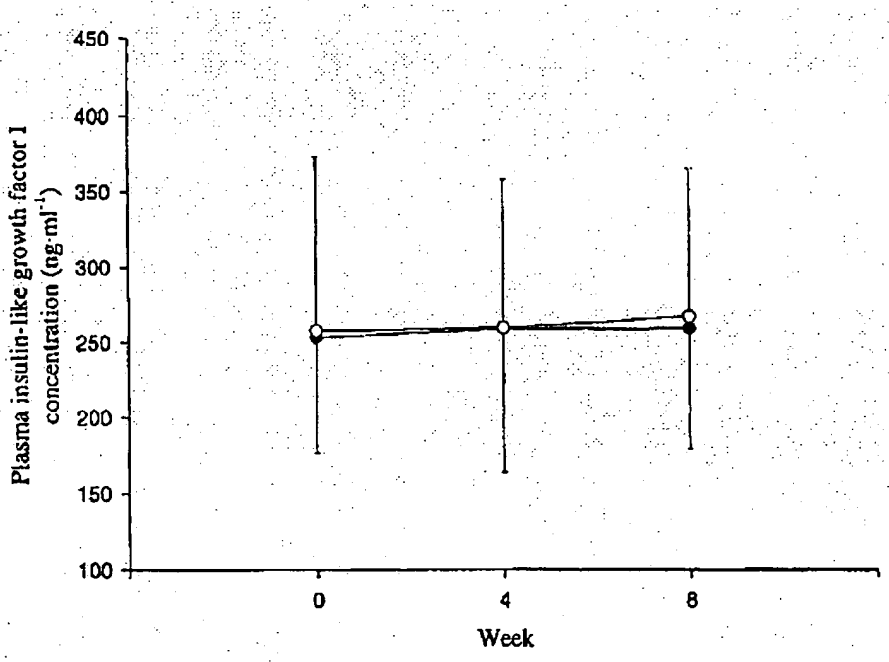

Fig. 1. Plasma insulin-like growth factor concentrations before and after 4 and 8 weeks of supplementation with concentrated bovine colostrum protein powder (●) or a concentrated whey protein placebo (○) during combined resistance and plyometric training (mean ± *s*).

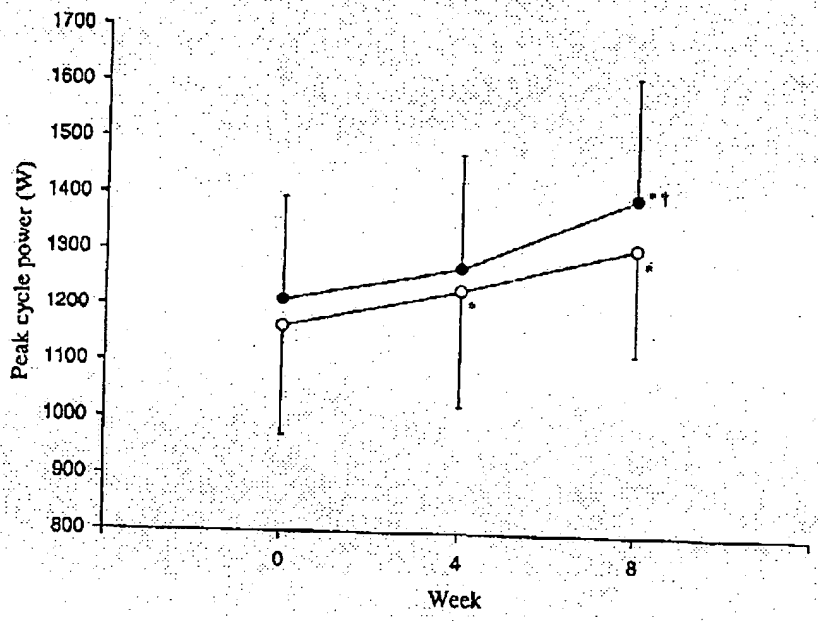

Fig. 2. Peak anaerobic cycle power during 10 s of maximal cycle exercise before and after 4 and 8 weeks of supplementation with concentrated bovine colostrum protein powder (●) or a concentrated whey protein placebo (○) during combined resistance and plyometric training (mean ± *s*). *Significantly different from week 0 ($P < 0.05$). †Significantly different from whey protein ($P < 0.01$).

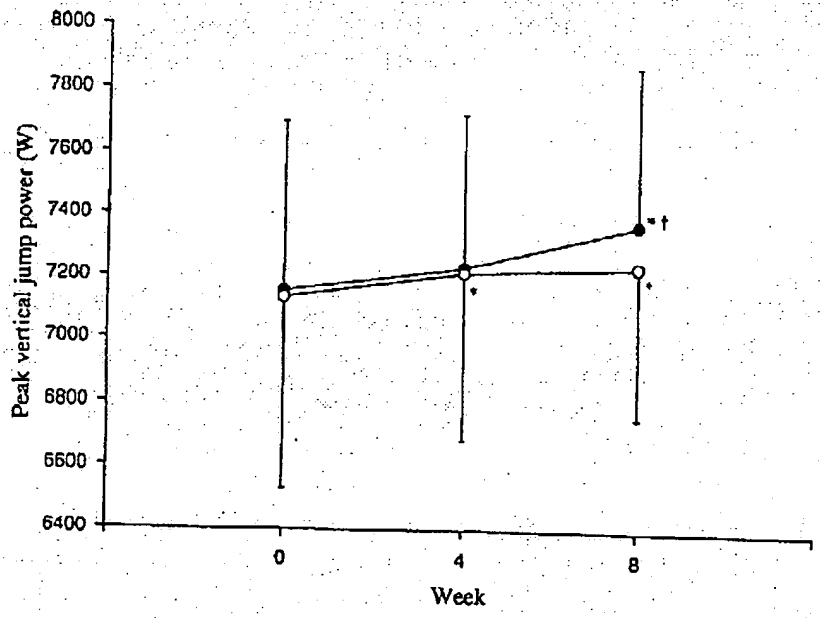

Fig. 3. Vertical jump power before and after 4 and 8 weeks of supplementation with concentrated bovine colostrum protein powder (●) or a concentrated whey protein placebo (○) during combined resistance and plyometric training (mean ± *s*). *Significantly different from week 0 ($P < 0.05$). †Significantly different from whey protein ($P < 0.01$).

differences in anaerobic work capacity at week 4 (bovine colostrum, 9.9±1.7 kJ; whey protein, 9.7±1.6 kJ; $P=0.70$) or at week 8 (bovine colostrum, 11.0±1.6 kJ; whey protein, 10.4±1.5 kJ; $P=0.17$).

Vertical jump power

The peak power outputs achieved during the vertical jump tests were significantly greater than those achieved during the cycle tests ($P<0.001$). However, the pattern of increase in peak vertical jump power was not different from that for peak cycle power ($P=0.85$). There was no difference in peak vertical jump power between the two groups at week 0 ($P=0.99$; Fig. 3). Peak vertical jump power did increase by a similar amount in both groups by week 4 ($P=0.99$; 95% CI = 6150 to 6158 W), but had increased significantly more in the bovine colostrum group by week 8 ($P<0.001$; 95% CI = 54 to 170 W), such that peak vertical jump power was significantly higher in this group by the end of the study period ($P<0.01$).

One-repetition maxima and training volume

The 1-RM data are reported in Table 1. The 1-RMs were not significantly different between groups at week 0 ($P>0.18$). The 1-RM for all exercises increased in both groups during the study period ($P<0.0001$), but there was no significant difference in the magnitude of any of the increases between groups ($P>0.08$). Accordingly, because training volumes were based on percentages of 1-RM, there were no differences in resistance training volumes completed by either group ($P=0.37$; Table 5). Nor were there any differences in training volumes for the non-resisted ($P=0.29$) or plyometric ($P>0.56$; Table 5) exercises between the two groups.

Dietary intake

There were no differences in daily energy, macronutrient or alcohol intakes between the two groups during the study period ($P>0.39$; Table 4).

Discussion

The major findings of the present study were that 8 weeks of supplementation with 60 g·day^{-1} of concentrated bovine colostrum protein powder during combined resistance and plyometric training resulted in significantly greater increases in peak vertical jump power and peak anaerobic cycle power compared with whey protein powder. However, bovine colostrum had no effect on alactic anaerobic work capacity, 1-RM or plasma IGF-I concentrations. The greater improvements in peak power output in the bovine colostrum group could not be attributed to differences in training or diet, since the training volumes completed and – apart from the different supplements taken – the dietary intakes of the two groups did not differ.

The present results indicate that the bovine colostrum supplement only provided a significantly greater improvement in peak vertical jump power and peak cycle power after 8 weeks of supplementation. There were no differences in peak vertical jump power or peak cycle power between the groups after only 4 weeks of supplementation. The reason for the delay before the supplement provided a benefit is not clear, but would account for why Mero *et al.* (1997) previously failed to demonstrate any effect of only 8 days of bovine colostrum supplementation on vertical jump performance. That there was an 8 week delay before an effect of the bovine colostrum supplement became evident is

Table 5. Training volumes completed during 8 weeks of supplementation with concentrated bovine colostrum protein powder (intact^{TM}) or concentrated whey protein powder during combined resistance and plyometric training (mean ± *s*)

		Completed by week 4	Completed by week 8
Resistance training (kg × 1000)	BC	216±45.6	450±84.5
	WP	200±61.3	424±121.5
Non-resisted exercises (repetitions)	BC	1722±204	3501±300
	WP	1656±249	3378±438
Plyometric training (km)	BC	11.5±1.1	22.8±1.8
	WP	11.2±1.6	22.5±2.8
Vertical jump (repetitions)	BC	112±11	221±18
	WP	112±13	220±24

Abbreviations: BC = concentrated bovine colostrums protein powder (intact^{TM}); WP = concentrated whey protein powder.

in line with the results of a previous study, which found that a bovine colostrum supplement had no effect on recovery from maximal endurance exercise after 4 weeks, but provided a significant improvement in recovery after 8 weeks of supplementation (Buckley *et al.*, 2002). Although the reason for the delayed effect of the supplement is not known, by the end of the 8 week supplementation period the bovine colostrum group had increased their peak vertical jump power by approximately 1.5% more than the whey protein group in our sample, and peak cycle power had increased by approximately 2.5% more, with both of these values being greater than the typical errors of measurement for the respective tests. The 95% confidence intervals for the differences in improvement between the two groups indicated that, for the average participant, bovine colostrum supplementation would provide somewhere between a 54 W (0.8%) and a 170 W (2.3%) greater improvement in peak vertical jump power, and between a 20 W (1.7%) and a 61 W (5.2%) greater improvement in peak cycle power. Given that the participants in the whey protein group improved their peak vertical jump power by only 1.6% after 8 weeks of training, the potential for the bovine colostrum supplement to provide an additional improvement of up to 2.3% would represent an important benefit. Similarly, given that peak cycle power had increased by 13.4% after 8 weeks in the whey protein group, the potential for bovine colostrum to provide an additional increase of up to 5.2% would also be considerable. However, given that the participant cohort used in the present study consisted of active males, it cannot necessarily be assumed that improvements of a similar magnitude would be obtained in well-trained or elite athletes, who would almost certainly have a level of physiological development that is closer to their genetic limits.

The peak power developed in the vertical jump tests was significantly greater than that developed during the cycle tests, probably reflecting the recruitment of a greater muscle mass in the vertical jump. In addition to the power developed by the legs, the upper body also develops significant vertical momentum during countermovement jumps, with the arm swing contributing approximately 10% to jump height (Luhtanen and Komi, 1978; Young *et al.*, 2001) and the trunk contributing a further 10% (Luhtanen and Komi, 1978). The power developed during a cycle test, on the other hand, relies predominantly on the power that can be developed by the legs. Despite the differences in the magnitude of the power developed, there was no statistical interaction between the improvements in peak power between the two tests, indicating that bovine colostrum exerted a similar effect on both peak vertical jump power and peak cycle power. Both the vertical jump and cycle tests provide a measure of anaerobic power, which is itself a function of the rate at which phosphagen stores within the skeletal muscles can be hydrolysed (Sawka *et al.*, 1980). Therefore, from the greater increases in both peak vertical jump power and peak cycle power, it would follow that bovine colostrum increased the rate of muscle phosphagen hydrolysis in both the upper and lower body musculature. Such an increase in muscular phosphagenolytic rate could have resulted from (1) increased cross-bridge cycling due to the recruitment of additional motor units or an increased contractile protein content, or (2) an increase in the specific activity of myosin ATPases due to a relative increase in the proportion of fast myosin heavy chains. Since bovine colostrum supplementation had no significant effect on 1-RMs in this study, or in a previous study by Antonio *et al.* (2001), and 1-RM provides a measure of muscular strength, which is itself a function of the number of actin–myosin cross-bridge attachments per half of a sarcomere acting in parallel (Garfinkel and Cafarelli, 1992), it is unlikely that bovine colostrum supplementation exerted its effect by increasing muscle contractile protein content or the recruitment of additional motor units. Instead, it is more likely that the increased phosphogenolytic rate may have resulted from an increase in the relative proportion of fast myosin heavy chains. Further support for this argument comes from the findings of a recent study from our laboratory, which showed that bovine colostrum supplementation increased buffer capacity in elite female rowers (Brinkworth *et al.*, 2002), since increases in buffer capacity are generally associated with adaptations in fast-twitch muscle fibres (Maughan *et al.*, 1997), which contain predominantly fast myosin isoforms. Future studies should investigate the effects of bovine colostrum supplementation on the expression of skeletal muscle contractile proteins and myosin heavy chain isoforms to enable a better understanding of the mechanism by which this supplement increases muscular power.

Although bovine colostrum supplementation increased peak anaerobic power, it had no significant effect on alactic anaerobic work capacity. Alactic anaerobic work capacity reflects the magnitude of change in phosphagen concentrations within the active skeletal muscles during exercise (Sawka *et al.*, 1980). Therefore, although the bovine colostrum supplement appeared to stimulate an increase in the rate of muscle phosphagen hydrolysis, it seemed to have no effect on the quantity of phosphagen hydrolysed. This implies that alactic anaerobic work capacity may have been limited by phosphagen availability rather than phosphagenolytic rate, and suggests that the combination of bovine colostrum supplementation with a strategy for increasing muscle phosphagen stores (e.g. creatine monohydrate supplementation) might provide a synergistic effect on anaerobic exercise performance. This proposition is supported by preliminary findings from a

recent study, which indicated that the combined ingestion of bovine colostrum and a supplement that contained creatine monohydrate during training resulted in greater increases in strength and/or power compared with the ingestion of the individual supplements alone (Kerksick *et al.*, 2001).

Mero and co-workers have previously shown that bovine colostrum supplementation increases serum IGF-I concentrations (Mero *et al.*, 1997, 2002), with the latter of these studies indicating that the additional IGF-I was unlikely to have been absorbed from the ingested colostrum supplement (Mero *et al.*, 2002). Regardless of the source of any additional IGF-I, an increase in circulating IGF-I concentration may be important for increasing muscular strength, since it was recently shown that increased circulating IGF-I concentrations may, at least in part, mediate the increases in 1-RM strength that result from resistance training (Borst *et al.*, 2001). However, neither the present study nor a previous study by Antonio *et al.* (2001) provided any evidence that bovine colostrum supplementation increases strength, because bovine colostrum had no effect on 1-RM. Furthermore, despite Mero *et al.* (1997, 2002) showing that bovine colostrum increases circulating IGF-I concentrations, neither the present study nor a number of previous studies (Buckley *et al.*, 2002; Coombes *et al.*, 2002; Kuipers *et al.*, 2002) have found any effect of bovine colostrum supplementation on circulating IGF-I concentrations. The difference in findings between these studies and those of Mero *et al.* (1997, 2002) may be related to the different durations of the blood sampling protocols used. Mero and co-workers used short supplementation periods of up to 2 weeks in both of their studies, whereas the other studies used longer supplementation periods of up to 8 weeks, and the first blood samples for assessing changes in circulating IGF-I concentrations were not drawn until at least 4 weeks after supplementation had begun. Therefore, the increases in IGF-I reported by Mero *et al.* (1997, 2002) may have been transient, and not detected in these other studies because of the 4 week interval between the assessment of circulating IGF-I concentrations before supplementation and their next assessment 4 weeks later. During this 4 week period, plasma IGF-I concentrations may have increased, before negative feedback mechanisms acted to return circulating concentrations to baseline. However, given that the present study found no effect of bovine colostrum on performance at week 4, and no effect on 1-RM overall, it is difficult to see how a transient increase in circulating IGF-I concentration during the first weeks of supplementation may mediate the supplement's performance effects. Further research should be directed at determining the kinetics of circulating IGF-I changes in response to bovine colostrum supplementation and how, if at all, these changes relate to improvements in physical performance.

The energy and protein contents of the two supplements used in the present study were similar, as were the total dietary energy and macronutrient intakes of the two supplement groups, suggesting that the differing performance outcomes resulted from the effect of some non-nutrient component in bovine colostrum, such as a growth factor or other mitogenic component. A similar finding was reported in a study conducted using neonatal pigs (Burrin *et al.*, 1995), which showed that skeletal muscle protein synthesis was increased in colostrum-fed piglets compared with formula-fed and/or milk-fed piglets despite no difference in macronutrient intakes between the three treatment groups. Therefore, although it appears that the physiological (Mero *et al.*, 1997, 2002; Antonio *et al.*, 2001; Brinkworth *et al.*, 2002) and/or performance-enhancing (Buckley *et al.*, 2002; Coombes *et al.*, 2002; Hofman *et al.*, 2002) effects of colostrum most likely result from the effect of some non-nutrient component, no studies to date have been able to identify the specific non-nutrient component(s) responsible. The present finding that bovine colostrum did not change the circulating IGF-I concentration, as well as data from animal studies that have shown that skeletal muscle protein synthesis is stimulated independently of changes in circulating IGF-I (Burrin *et al.*, 1992, 1995), suggest that IGF-I is unlikely to mediate the effects of bovine colostrum. However, bovine colostrum contains several other peptide growth factors (Donovan and Odle, 1994; Pakkanen and Aalto, 1997) and as yet unidentified mitogenic components (Belford *et al.*, 1997) that may be responsible for the reported effects of this supplement. As the physiological and performance effects of bovine colostrum supplementation become better characterized, it should be possible to refine our understanding of the possible mechanisms of action of this supplement, which may assist in identifying the colostral component(s) responsible.

In conclusion, supplementation with concentrated bovine colostrum protein powder during 8 weeks of combined resistance and plyometric training increased anaerobic power compared with a whey protein placebo, but had no effect on alactic anaerobic work capacity, 1-RM or plasma IGF-I concentrations. These findings suggest that bovine colostrum might be a useful supplement for improving athletic performance in events that rely on the development of high anaerobic power, but further research is required to determine the mechanism of action of this supplement.

Acknowledgements

The authors gratefully acknowledge Andrew Modra and Simon Gunn for their assistance with the collection of data

for this study, and Dr Phillip Owens from the University of Adelaide for performing the IGF-I assays. This work was supported by a grant from Numico Research (Australia) Pty Ltd, Adelaide, Australia.

References

Antonio, J., Sanders, M. and Van Gammeren, D. (2001). The effects of bovine colostrum supplementation on body composition and exercise performance in active men and women. *Nutrition*, 17, 243–247.

Belford, D., Rogers, M.-L., Francis, G., Payne, C., Ballard, F. and Goddard, C. (1997). Platelet-derived growth factor, insulin-like growth factors, fibroblast growth factors and transforming growth factor b do not account for the cell growth activity present in bovine milk. *Journal of Endocrinology*, 154, 45–55.

Borst, S., De Hoyos, D., Garzarella, L., Vincent, K., Pollock, B., Lowenthal, D. and Pollock, M. (2001). Effects of resistance training on insulin-like growth factor-I and IGF binding proteins. *Medicine and Science in Sports and Exercise*, 33, 648–653.

Brinkworth, G., Buckley, J., Bourdon, P., Gulbin, J. and David, A. (2002). Bovine colostrum supplementation improves buffer capacity, but not rowing performance in elite female rowers. *International Journal of Sports Nutrition*, 12, 349–363.

Buckley, J., Abbott, M., Brinkworth, G. and Whyte, P. (2002). Bovine colostrum supplementation during endurance running training improves recovery, but not performance. *Journal of Science and Medicine in Sport*, 5, 65–79.

Burrin, D., Shulman, R., Reeds, P., Davis, T. and Gravitt, K. (1992). Porcine colostrum and milk stimulate visceral organ and skeletal muscle protein synthesis in newborn pigs. *Journal of Nutrition*, 122, 1205–1213.

Burrin, D., Davis, T., Ebner, S., Schoknecht, P., Fiorotto, M., Reeds, P. and McAvoy, S. (1995). Nutrient-independent and nutrient-dependent factors stimulate protein synthesis in colostrum-fed newborn pigs. *Pediatric Research*, 37, 593–599.

Coombes, J., Conacher, M., Austen, S. and Marshall, P. (2002). Dose effects of oral bovine colostrum on physical work capacity in cyclists. *Medicine and Science in Sports and Exercise*, 34, 1184–1188.

Donovan, S.M. and Odle, J. (1994). Growth factors in milk as mediators of infant development. *Annual Review of Nutrition*, 14, 147–167.

Ellis, L., Gastin, P., Lawrence, S., Savage, B., Buckeridge, A., Stapff, A., Tumilty, D., Quinn, A., Woolford, S. and Young, W. (2000). Protocols for the physiological assessment of team sport players. In *Physiological Tests for Elite Athletes* (edited by C. Gore), pp. 128–144. Champaign, IL: Human Kinetics.

Fiorotto, M., Davis, T., Reeds, P. and Burrin, D. (2000). Nonnutritive factors in colostrum enhance myofibrillar protein synthesis in the newborn pig. *Pediatric Research*, 48, 511–517.

Garfinkel, S. and Cafarelli, E. (1992). Relative changes in maximal force, EMG, and muscle cross-sectional area after isometric training. *Medicine and Science in Sports and Exercise*, 24, 1220–1227.

Harman, E., Rosenstein, M., Frykman, P., Rosenstein, R. and Kraemer, W. (1991). Estimation of human power ourput from vertical jump. *Journal of Applied Sport Science Research*, 5, 116–120.

Hofman, Z., Smeets, R., Verlaan, G., vd Lugt, R. and Verstappen, P. (2002). The effect of bovine colostrum supplementation on exercise performance in elite field hockey players. *International Journal of Sports Nutrition*, 12, 461–469.

Jackson, M. (1991). *The 12345+ Food and Nutrition Plan*. Adelaide: Anti Cancer Foundation of South Australia.

Kerksick, C., Kreider, R., Rasmussen, C., Lancaster, L., Starks, M., Greenwood, M., Milnor, P., Almada, A. and Earnest, C. (2001). Effects of bovine colostrum supplementation on training adaptations II: performance. *Journal of the Federation of the American Society for Experimental Biology*, 15, LB58.

Kuipers, H., van Breda, E., Verlaan, G. and Smeets, R. (2002). Effects of oral bovine colostrum supplementation on serum insulin-like growth factor I levels. *Nutrition*, 18, 566–567.

Luhtanen, P. and Komi, P. (1978). Segmental contribution to forces in vertical jump. *European Journal of Applied Physiology*, 38, 181–188.

Maughan, R.J., Gleeson, M. and Greenhaff, P.L. (1997). *Biochemistry of Exercise and Training*. New York: Oxford University Press.

Mero, A., Miikkulainen, H., Riski, J., Pakkanen, R., Aalto, J. and Takala, T. (1997). Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training. *Journal of Applied Physiology*, 83, 1144–1151.

Mero, A., Kahkonen, J., Nykanen, T., Parviainen, T., Jokinen, I., Takala, T., Nikula, T., Rasi, S. and Leppaluoto, J. (2002). IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training. *Journal of Applied Physiology*, 93, 732–739.

Norton, K. and Olds, T. (1996). *Anthropometrica*. Sydney: University of New South Wales Press.

Olds, T. and Norton, K. (1999). *Pre-exercise Health Screening Guide*. Champaign, IL: Human Kinetics.

Owens, P.C., Johnson, R.J., Campbell, R.G. and Ballard, F.J. (1990). Growth hormone increases insulin-like growth factor-I (IGF-I) and decreases IGF-II in plasma of growing pigs. *Journal of Endocrinology*, 124, 269–275.

Owens, J.A., Kind, K.L., Carbone, F., Robinson, J.S. and Owens, P.C. (1994). Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. *Journal of Endocrinology*, 140, 5–13.

Pakkanen, R. and Aalto, J. (1997). Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal*, 7, 285–297.

Sawka, M., Tahamont, M., Fitzgerald, P., Miles, D. and Knowlton, R. (1980). Alactic capacity and power. *European Journal of Applied Physiology*, 45, 109–116.

Scott, C.D. and Baxter, R.C. (1986). Production of insulin-like growth factor I and its binding protein in rat hepatocytes cultured from diabetic and insulin-treated diabetic rats. *Endocrinology*, 119, 2346–2352.

Telford, R., Minikin, B., Hahn, A. and Hopper, L. (1989). A simple method for the assessment of general fitness: the tri-level profile. *Australian Journal of Science and Medicine in Sport*, 21, 6–9.

Young, W., MacDonald, C. and Flowers, M. (2001). Validity of double- and single-leg vertical jumps as tests of leg extensor muscle function. *Journal of Strength and Conditioning Research*, 15, 6–11.

2692.3

Dose effects of oral bovine colostrum on physical work capacity in cyclists

JEFF S. COOMBES[1], MICHAEL CONACHER[2], SIMON K. AUSTEN[1], and PHILIP A. MARSHALL[3]

[1]*School of Human Movement Studies, University of Queensland, St Lucia, AUSTRALIA;* [2]*Centre for Human Movement, University of Tasmania, Launceston, TASMANIA;* [3]*Numico Research Australia, Oakden, SOUTH AUSTRALIA*

ABSTRACT

J. S. COOMBES, M. CONACHER, S. K. AUSTEN, P. A. MARSHALL. Dose effects of oral bovine colostrum on physical work capacity in cyclists. *Med. Sci. Sports Exerc.*, Vol. 34, No. 7, pp. 1184–1188, 2002. Purpose: There is interest in the potential long-term use of dietary supplementation with bovine colostrum to enhance exercise performance. The purpose of the present study was to determine the dose effects of bovine colostrum on cycling performance. Methods: Forty-two competitive cyclists were randomly divided into three groups and required to consume either 20 g/d bovine colostrum + 40 g whey protein concentrate (wpc), 60 g of bovine colostrum, or 60 g of wpc (placebo). Two measures were used to assess performance before (pre-) and after (post-) an 8-wk supplementation period. The first measure required subjects to complete two $\dot{V}O_{2max}$ tests separated by 20 min with the amount of work completed in the second test used to evaluate performance. The second performance measure was the time to complete a work-based time trial following a 2-h cycle at 65% $\dot{V}O_{2max}$. Subjects were required to maintain their regular training and keep a food and training diary over the study period. Results: After supplementation, the performance enhancement in Measure One was not statistically significantly different in the colostrum groups compared to the placebo group (placebo = 3.4%, 20 g = 4.0%, 60 g = 3.9%; 95% confidence interval (CI) for differences, ±1.8%, $P > 0.05$). In performance Measure Two subjects in the 20 g and 60 g groups completed the time trial significantly ($P < 0.05$) faster post supplement compared to pre supplement (improvements in performance times, placebo = 37 s, 20 g = 158 s, 60 g = 134 s; 95% CI for differences, 47 s). Conclusion: Oral bovine colostrum supplementation at 20 g or 60 g/d provided a small but significant improvement in time trial performance in cyclists after a 2-h ride at 65% $\dot{V}O_{2max}$.
Key Words: EXERCISE PERFORMANCE, DIETARY SUPPLEMENTATION, IGF-1, CYCLING TIME TRIAL

Colostrum is the milk produced by the mammary glands of all mammals, including humans, during the first 72 h after birth. It is fed to their newborns to provide essential nutrients and bioactive components including growth factors, immunoglobulins, vitamins, minerals and amino acids (7). Currently bovine colostrum is being marketed as a nutritional ergogenic aid, although there is limited research investigating the effects of colostrum supplementation on exercise performance.

A recent study reported that 60 g/d bovine colostrum supplementation for 8 wk improved the ability to perform a second bout of maximal exercise following a relatively short period of recovery from a prior bout of maximal exercise (1). Subjects were required to complete two treadmill $\dot{V}O_{2max}$ running tests separated by 20 min. The amount of work completed in the second $\dot{V}O_{2max}$ test compared with the first was significantly improved after the study period in the group supplemented with colostrum. The same protocol with a cycle ergometer and a second smaller dose (20 g) of colostrum is used in this study.

0195-9131/02/3407-1184/$3.00/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Submitted for publication March 2001.
Accepted for publication March 2002.

METHODS

Subjects

All testing occurred in the temperature and humidity controlled Human Performance Laboratory at the University of Tasmania and at the Tasmanian Institute of Sport. Written informed consent was obtained from 42 competitive male cyclists. Fourteen of the subjects were withdrawn from the study due to noncompliance with either the supplementation or training; their data are not presented. The physical characteristics (mean ± SD) of the remaining 28 subjects were: age, 30 ± 10 yr; body mass, 74 ± 21 kg; sum of seven skinfolds, 67 ± 48 mm; $\dot{V}O_{2max}$, 61 ± 9 mL·kg^{-1}·min^{-1}. Screening information was obtained to ensure that subjects met the following criteria: 1) no history of lactose or cow's milk protein intolerance, 2) not taking nutritional supplements, 3) no history of vascular, metabolic or respiratory disease, and 4) no chronic health problems. Subjects attended a briefing session before any experimentation to ensure an understanding of the testing procedures and the benefits/risks of the study. Emphasis was placed on subject preparation for the laboratory tests. Specific instructions were given to the subjects concerning their diet and training before the testing sessions. Subjects were required to abstain from exercise for 2 d preceding the sessions. For the first session, subjects were required to document their nutritional and fluid intake in the 24 h before the test. For all remaining lab sessions, subjects were required to approximate this 24-h diet before reporting to the laboratory. At the start of each

test session subjects documented their ability to adhere to these instructions. The protocol was approved by the University of Tasmania's Ethics Committee.

Design

A randomized, double-blind, placebo (Alacen™, Fonterra Co-operative Group Ltd., New Zealand) controlled design was used to evaluate the dose effects of colostrum supplementation on physical work capacity in cyclists. Subjects were randomly allocated to one of three groups: 1. placebo control group supplemented with 60 g/d oral whey-protein powder (placebo, $N = 10$), 2. high dose colostrum group supplemented with 60 g/d oral bovine colostrum (60 g, intact*), ($N = 9$) or 3. low dose colostrum group supplemented with 20 g/d oral bovine colostrum (intact) and 40 g/d whey-protein powder (20 g), ($N = 9$). Each subject completed two performance tests before and after an eight week supplementation period.

Performance testing

Performance Measure One. On the first visit, subjects reported to the laboratory in a rested condition, having eaten a substantial meal and abstained from caffeine, drugs, alcohol, and cigarettes 4 h before testing. Measurements of weight and skinfolds from 7 sites (bicep, tricep, subscapular, suprailiac, abdominal, quadriceps, and medial calf) were obtained before the commencement of performance Measure One. Following a 10 min warm up and stretching exercises, subjects completed two identical-protocol $\dot{V}O_{2max}$ tests separated by 20 min. The protocol required subjects to maintain a self-selected cadence of between 70 and 100 rpm on a Lode cycling ergometer with an initial load of 100 W for 3 min, increased to 200 W for 3 min, and then 50 W every 3 min thereafter until exhaustion. Expired air was analyzed throughout the test using a Quinton Metabolic Cart (Quinton, QMC, Bothwell, WA). Subjects were required to breathe room air through a Hans Rudolph 2700 valve (Hans Rudolph, inc., Kansas City, MO) with expired air traveling via 3.5 cm tubing to the mixing chamber of the QMC. Both oxygen and carbon dioxide analyzers were calibrated before and verified after each test with alpha standard gases (BOC Gases, Australia) of two known concentrations (a = 14.2% O_2 and 3.4% CO_2 and b = 18.4% O_2 and 5.1% CO_2). The pneumotach was calibrated before and verified after each test session using a 3 L calibration syringe (Hans Rudolph). For the 20 min recovery period between the two $\dot{V}O_{2max}$ tests, the mouthpiece and headgear were removed while subjects were given a 3 min cool down on the ergometer followed by stretching and were instructed to remain active by walking around the laboratory. Heart rate was recorded throughout the $\dot{V}O_{2max}$ tests using a Sportstester PE3000 system (Polar Electro, Kempele, Finland).

Performance Measure Two. Two days after the first performance test, subjects returned to the laboratory in a similar state of physical preparation and at the same time of the day for Performance Measure Two. The test consisted of a 2 h performance ride on the lode cycle ergometer at 65% of their maximal heart rate, determined as the highest heart rate obtained during Performance Measure One. During the post-supplementation test their maximal heart rate was determined from their post-supplementation Performance Measure One $\dot{V}O_{2max}$ tests. Subjects were instructed to prepare for the ride as they would prepare for a 100 km road race by bringing food and drink, which they would usually consume during the race, to the laboratory. Subjects were permitted and encouraged to consume food and fluids *ad libitum* with additional supplies available on request. Nutrient and fluid intake was recorded during the ride. Heart rate was measured throughout the 2 h ride and time trial using a Sportstester system. At 5 min intervals during the 2 h ride, the intensity was adjusted to maintain the desired heart rate. The 2 h ride was immediately followed by a workload based time trial where each subject was required to complete 2.8 kJ/kg of work as fast as possible. The time for this trial was used as the second performance measure.

At the completion of the second visit subjects received a box of their respective supplement containing 20 g sachets labeled days 1 through 56. Each sachet contained a morning and evening packet. Subjects were required to consume a morning dose of 20 g with 85 mL warm water and 40 mL skim milk, and an evening dose of 40 g with 170 mL warm water and 80 mL skim milk. Subjects were also given a diet and training diary with instructions on how to complete it.

After 8 wk of supplementation, subjects reported back to the laboratory for re-testing (Performance Measure One and two days later for Performance Measure Two).

Training/Diet Diaries

Subjects were required to keep a daily training and diet diary for the 8 wk supplementation period. Dietary information was analyzed using Foodworks (Xyris Software, Queensland, Australia). The diet diary provided daily averages for energy, macro-, and micronutrient intake. Training volume was calculated as an average of minutes trained per day.

Blood Analysis

Plasma Insulin-like growth factor I. In response to the finding that bovine colostrum supplementation increased serum insulin-like growth factor I (IGF-1) concentrations during training (4), we measured plasma IGF-1 concentrations over the supplementation period. Blood was collected from each subject before Performance Measure Two pre- and post-supplementation. Plasma IGF-1 was measured by radioimmunoassay after separation from binding proteins by high performance size exclusion liquid chromotography at pH 2.5 according to the method of Scott and Baxter (9) as modified by Owens (6). Recombinant *h*IGF-1 (GroPep Pty Ltd, Adelaide, Australia) was used, and the radioligand was prepared to a specific activity of Ci/g with chloranane-T and NaI^{125} (Amersham Biosciences, Uppsala, Sweden). Antiserum to human IGF-1 was raised in rabbit (10). Samples

TABLE 1. Comparison of body mass, body composition, aerobic capacity (peak value obtained from both trials), and plasma IGF-1 between the three groups pre- and post-supplementation.

	Placebo (N = 10)		20 g (N = 9)		60 g (N = 9)	
	Pre	Post	Pre	Post	Pre	Post
Body mass (kg)	72 ± 11	73 ± 10	73 ± 7	73 ± 7	75 ± 17	75 ± 17
Sum of 7 skinfolds (mm)	70 ± 28	68 ± 22	69 ± 19	64 ± 18	64 ± 32	58 ± 20
VO_{2max} (mL/kg/min)	59 ± 5	56 ± 2	62 ± 4	59 ± 4	63.2 ± 5	60 ± 7
Plasma IGF-1 (ng/mL)	157 ± 79	177 ± 73	239 ± 39	253 ± 32	201 ± 71	173 ± 45

Values are mean ± SD.

were stripped of IGF-binding proteins in four chromatography sessions. The fraction containing IGF-I routinely eluted from the size exclusion HPLC column between 8.25 and 10.5 min after injection of the acidified plasma samples. The recovery from the column estimated from injections of [^{125}I]-iodo-IGF-I was 85 ± 1% (mean ± SD, N = 5). Samples were measured in triplicate in a total of two assays, with each of the triplicates being included in the same chromatography session and measured in the same assay. The average minimal detectable concentration was 16 ng·mL^{-1} (range 9 to 21 ng·mL^{-1}) and the average half-maximal response in the assay was produced by a sample containing 232 ng·mL^{-1} (range 225 to 234 ng·mL^{-1}). For replicates of a quality control plasma specimen, whose average IGF-I concentration was determined to be 50 ng·mL^{-1} after being measured four times in each of the assays, the average within assay coefficient of variation was 4%, and the between assay coefficient of variation was 11%.

Means and standard deviations are used to represent the average and typical spread of values. The precision of the estimates for outcome statistics are shown as 95% confidence limits (the likely range of the true value). All data were analyzed using analysis of variance (ANOVA) with a Fisher *post hoc* test. Pre vs post repeated measures factor was adjusted with Greenhouse-Geisser epsilon. $P < 0.05$ was regarded as statistically significant.

RESULTS

There were no significant differences in changes in body mass, body composition, aerobic capacity (peak value obtained from both trials), or plasma IGF-1 between the three groups pre- and post-supplementation (Tab. 1). Table 2 shows the daily averages for nutrient intake and training volume for each group. There were no significant differences between groups for any of the variables.

Results for Performance Measure One are shown in Table 3 and Figure 1. There were no significant differences between groups or within groups (pre- vs post-supplementation) for any of the absolute values for work completed during each ride or the average of both rides. When the work from the second VO_{2max} test is expressed as a percentage of the work completed in the first VO_{2max} test, and pre-supplementation values are compared with post-supplementation, subjects improved by 3.4%, 4.0%, and 3.9% in the placebo, 20 g, and 60 g groups, respectively (Fig. 1). In each case the difference between the changes was not statistically significant (95% CI for differences, ±1.8%).

Nutritional and fluid intake was recorded during the Second Performance Measure. There were no significant differences (data not shown) between groups or within groups (pre- vs post-supplementation) in nutrient or fluid intake during the ride. In the Second Performance Measure (Tab. 4 and Fig. 2), there were increases of 37 s, 134 s, and 158 s in the placebo, 20 g, and 60 g groups, respectively (95% CI for differences, ±47 s). The performance improvements in the 20 g and 60 g groups were both significantly greater ($P <$ 0.05) than that in the placebo group.

TABLE 2. Comparison of the nutrient intake and training volume of the three groups throughout the supplementation period.

Daily Averages	Placebo (N = 10)	20 g (N = 9)	60 g (N = 9)
Energy (kJ/kg)	154 ± 53	149 ± 31	149 ± 41
Carbohydrate (%)	54.7 ± 6.3	52.7 ± 6.5	50.1 ± 9.3
Fat (%)	26.3 ± 5.4	26.7 ± 5.1	29.5 ± 7.4
Protein (%)	15.8 ± 2.1	16.6 ± 3.1	18 ± 3.4
Training volume (min/d)	92 ± 32	85 ± 27	87 ± 64

Values are mean ± SD.

DISCUSSION

To evaluate the dose effects of colostrum supplementation on cycling work capacity, two performance measures were chosen. The major finding of this study was that in one performance measure there was a significant improvement in cycling work capacity in those subjects consuming either 20 g or 60 g/d of bovine colostrum. Although we cannot discount the possibility that this was a chance finding, importantly the 95% confidence intervals for the differences

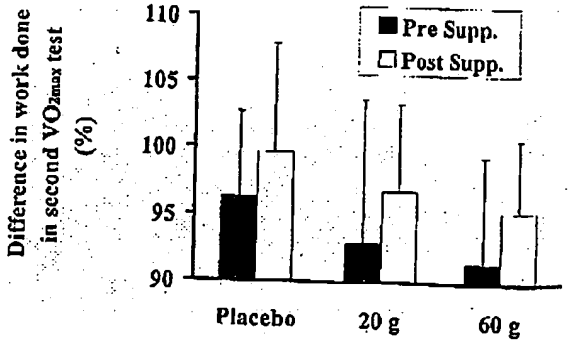

FIGURE 1—Performance measure one. Subjects were required to complete two VO_{2max} tests separated by 20 min. The amount of work completed in the second ride as a percentage of the first is shown for the three groups pre- and post-supplementation. Means (±SD).

TABLE 3. Data from performance measure one

	Pre-supplementation			Post-supplementation			Improvement
	Work Completed in 1st $\dot{V}O_{2max}$ Test (kJ)	Work Completed in 2nd $\dot{V}O_{2max}$ Test (kJ)	Average Work Completed in 2 Tests (kJ)	Work Completed in 1 $\dot{V}O_{2max}$ Test (kJ)	Work Completed in 2nd $\dot{V}O_{2max}$ Test (kJ)	Average Work Completed in 2 Tests (kJ)	Change in the Average Work Completed From Pre- to Post-Supplementation (kJ)
Placebo (N = 10)	295 ± 99	279 ± 83	287 ± 88	291 ± 89	285 ± 71	288 ± 78	−2 ± 20
20 g (N = 9)	395 ± 72	367 ± 81	381 ± 74	387 ± 65	374 ± 66	381 ± 62	1 ± 23
60 g (N = 9)	394 ± 77	362 ± 87	378 ± 78	411 ± 104	390 ± 86	400 ± 89	10 ± 29

Values are mean ± SD.

in improvements in performance times did not overlap (placebo = 37 s, 20 g = 158 s, 60 g = 134 s; 95% CI for differences, 47 s)

The present finding that 8 wk of oral bovine colostrum supplementation improved the amount of work completed after a 2 h cycle at 65% $\dot{V}O_{2max}$ is consistent with Buckley et al. (1) who used treadmill running. In this study, an 8 wk supplementation protocol of 60 g/d of colostrum was used. The present study indicates that similar performance benefits may be obtained with a smaller (20 g) dose.

Performance was not improved in Performance Measure One, which required subjects to complete two $\dot{V}O_{2max}$ tests separated by 20 min with the amount of work completed in the second test used as the performance determinant. This design is similar to that used by Buckley who reported a greater amount of work completed in the second treadmill test after 8 wk of colostrum supplementation. A possible explanation for the different findings in the present study and that of Buckley is the mode of the exercise test. Cyclists are accustomed to events of longer duration compared with runners. The incremental short term design (~ 20 min) of the $\dot{V}O_{2max}$ protocols used in Performance Measure One may not have been specific to the demands of a typical cycling event, and this test may be more suitable for assessing running performance. The second performance measure was designed to approximate the demands of a 100 km time trial and may be viewed as a more appropriate exercise test for cycling. Again, it was this second performance measure that showed a significant improvement in cycling performance in subjects consuming both 20 g and 60 g/d of colostrum.

A number of mechanisms may explain the action of bovine colostrum that caused the performance enhancement. Mero et al. (4) reported that colostrum supplementation increases circulating IGF-1. As bovine IGF-1 shares 100% homology with human IGF-1, it was postulated that an increased circulating IGF-1 would improve anabolic processes post-recovery by causing a greater overall adaptation to training and resultant observed performance enhancement. Consistent with the findings of Buckley, the present study showed no difference in plasma IGF-1 levels between the three groups. A likely explanation for the contradictory findings to Mero is the type of assay used. Mero used a radioimmunoassay that measures both the IGF-1 and its associated binding protein. A more appropriate and accepted procedure for plasma IGF-1 was used in the present study to first remove the binding protein before measuring IGF-1 (6).

A second mechanistic hypothesis is that colostrum supplementation enhances nutrient absorption from the small intestine. IGF-1 and epidermal growth factor (EGF) in the small intestine have been shown to stimulate gastrointestinal mucosal growth and brush border enzyme activity when given to suckling animals (2,5). Furthermore, it is well established that in adult animals administration of IGF-1 increases intestinal mucosal weight, protein and DNA content, villus height, and epithelial proliferation and function (3,8). We postulate that bovine colostrum supplementation improves small intestine function and nutrient absorption leading to enhanced nutrient availability to the recovering muscle cells. The enhanced nutrient availability may promote recovery after training by accelerating the repair of injured muscles, leading to a greater overall adaptation to training. Testing these postulates would provide an interesting area for future research.

A small but nonsignificant decrease was observed in $\dot{V}O_{2max}$ in each of the three groups over the course of the trial. This was due to the periodization of the cyclists training. All subjects tested were competitive cyclists who completed their pre-supplementation $\dot{V}O_{2max}$ test at the end of the road season when training volume was the highest. After these tests, the cyclists began preparing for the summer track season when training volume decreased due to a focus on power and strength work. They completed their post-supplementation $\dot{V}O_{2max}$ tests leading into the track season.

The significant improvements in time trial performance in the colostrum groups, added to the small decrease in $\dot{V}O_{2max}$ over the supplementation period, meant that subjects were working at a higher relative intensity during the post-supplementation time trial. We suspect that this was because of

TABLE 4. Data from performance measure two.

	Pre-supplementation Performance Ride Time (s)	Post-supplementation Performance Ride Time (s)	Improvement (s)	Improvement (%)
Placebo (N = 10)	819 ± 185	782 ± 93	37 ± 108	4 ± 2
20 g (N = 9)	826 ± 91	668 ± 78	158 ± 84*	19 ± 4*
60 g (N = 9)	825 ± 105	691 ± 31	134 ± 52*	16 ± 2*

Values are mean ± SD.
* Significantly greater than placebo ($P < 0.05$).

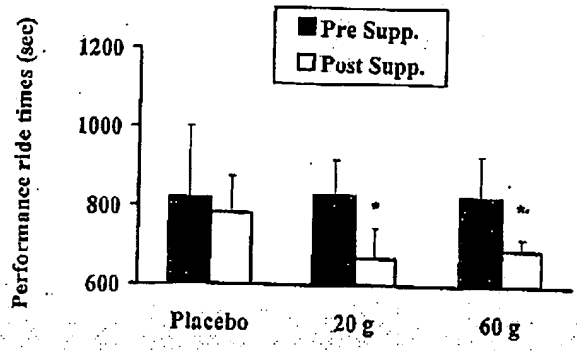

FIGURE 2—Performance measure two. Subjects were required to cycle for 2 h at 65% $\dot{V}O_{2max}$ then complete a work-based time trial. The time trial times for the three groups pre- and post-supplementation are shown. Means (±SD).* significantly less than placebo ($P < 0.05$).

the aforementioned change in training to high intensity work. Training diaries support this postulate with subjects documenting more hill and track training leading up to the post-supplementation testing. This training may have increased anaerobic threshold enabling subjects to work at a higher relative intensity post-supplementation.

One of the major strengths of this study was the research design. To ensure supplement and training compliance over the 8 wk supplementation period, subjects were monitored for nutritional intake and training volume, and a number of subjects were excluded from the study owing to their inability to maintain training volume or product adherence. Analysis of diet and training diaries showed that there were no differences between groups in the total energy intake, the composition of macronutrients, or the training volume.

In summary, the purpose of the study was to determine the dose effects of oral bovine colostrum supplementation on physical work capacity in cyclists using two performance tests. Colostrum supplementation failed to improve the ability to perform a second maximal work bout after a relatively short rest period, following a prior maximal work bout. In the second test, after a 2 h ride at 65% $\dot{V}O_{2max}$ two different colostrum supplement doses (20 g or 60 g/d) were associated with small but worthwhile enhanced performance in a work based time trial.

The authors thank Phillip Owens for analyzing plasma IGF-I, Tammy Ebert at the Tasmanian Institute of Sport for assistance with data collection, and the subjects for their patience and cooperation throughout the study.

* Intact™ is a registered trademark by Numico Research Australia for its concentrated colostrum protein (Australian Patents 644468, 668033, New Zealand Patents 239466, 260568).

Address for correspondence: Jeff Coombes, PhD, School of Human Movement Studies, Rm 520 Connell Bldg., University of Queensland, St Lucia, QLD, 4072, Australia; E-mail: jcoombes@hms.uq.edu.au

REFERENCES

1. Buckley, J., M. Abbott, S. Martin, G. Brinkworth, and P. Whyte. Effect of oral bovine colostrum supplement on running performance. *J. Sci. Med. Sport.* 5:65–79, 2002.
2. Burrin, D. G., T. J. Wester, T. A. Davis, S. Amick, and J. P. Heath. Orally administered IGF-I increases intestinal mucosal growth in formula-fed neonatal pigs. *Am. J. Physiol.* 270:R1085–R1091, 1996.
3. Lemmey, A. B., A. A. Martin, L. C. Read, F. M. Tomas, P. C. Owens, and F. J. Ballard. Igf-I, and the truncated analogue des-(1–3)IGF-I enhance growth in rats after gut resection. *Am. J. Physiol.* 260:E213–219, 1991.
4. Mero, A., H. Miikkulainen, J. Riski, R. Pakkanen, J. Aalto, and T. Takala. Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training. *J. Appl. Physiol.* 83:1144–1151, 1997.
5. Opleta-Madsen, K., J. B. Meddings, and D. G. Gall. Epidermal growth factor and postnatal development of intestinal transport and membrane structure. *Pediatr Res.* 30:342–350, 1991.
6. Owens, J. A., K. L. Kind, P. Carbone, J. S. Robinson, and P. C. Owens. Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. *J. Endocrinol.* 140:5–13, 1994.
7. Oyenivi, O. O., and A. G. Hunter. Colostral constituents including immunoglobulins in the first three milkings postpartum. *J. Dairy Sci.* 61:44–48, 1978.
8. Peterson, C. A., D. M. Ney, P. S. Hinton, and H. V. Carey. Beneficial effects of insulin-like growth factor I on epithelial structure and function in parenterally fed rat jejunum. *Gastroenterology.* 111:1501–1508, 1996.
9. Scott, C. D., and R. C. Baxter. Production of insulin-like growth factor I and its binding protein in rat hepatocytes cultured from diabetic and insulin-treated diabetic rats. *Endocrinology.* 119: 2346–2352, 1986.
10. Wolf, E., P. M. Jehle, M. M. Weber, et al. Human insulin-like growth factor 1 (IGF-I) produced in the mammary glands of transgenic rabbits: yield, receptor binding, mitogenic activity, and effects on IGF-binding proteins. *Endocrinology.* 138:307–313., 1997.

APPLIED NUTRITIONAL INVESTIGATION

Effects of Oral Bovine Colostrum Supplementation on Serum Insulin-like Growth Factor-I Levels

Harm Kuipers, MD, PhD, E. van Breda, PhD, G. Verlaan, MSc, and R. Smeets, PhD

From Maastricht University, Maastricht, The Netherlands; and Numico Research, Wageningen, The Netherlands

OBJECTIVES: We investigated whether supplementation with 60 g/d of bovine colostrum affects blood levels of insulin-like growth factor-I (IGF-I) and IGF binding protein-3 in relation to doping testing. Nine endurance-trained men ingested 60 g/d of bovine colostrum for 4 wk.

METHODS: Blood and urine were sampled before starting supplementation. After 4 wk urine and blood samples were taken after an overnight fast and 2 h after ingestion of the last portion to study possible acute effects.

RESULTS: Blood IGF-I levels before supplementation were (mean ± standard deviation) 31 ± 13 nM/L, and no acute effects were observed after 4 wk of supplementation (33 ± 9 nM/L). Levels of IGF-binding protein-3 were 136 ± 11 nM/L before supplementation and 135 ± 16 nM/L after 4 wk of supplementation. Two hours after ingestion of the last portion, the level of IGF binding protein-3 was 131 ± 19 nM/L, which was not different from baseline values. Drug testing in a laboratory accredited by the International Olympic Committee did not show any forbidden substance before or after 4 wk of supplementation.

CONCLUSIONS: Daily supplementation with 60 g of bovine colostrum for 4 wk does not change blood IGF-I or IGF binding protein-3 levels and does not elicit positive results on drug tests. *Nutrition* 2002; 18:566–567. ©Elsevier Science Inc. 2002

KEY WORDS: colostrum supplementation, growth factors, doping test

INTRODUCTION

Oral nutritional supplementation of colostrum, the first milk secreted by cows after calving, has long been advocated as promoting health. In the late 1980s it became an important nutritional supplement for athletes. Although it has not been established unequivocally that colostrum enhances performance in humans, athletes take oral bovine colostrum in an attempt to use all possible legal means to enhance performance. The interest in this supplement recently has regained momentum.

Colostrum is designed by nature as a substance that protects a newborn's immune system and provides passive immunity against a host of microorganisms. It also assists the body with protein synthesis, muscle building, and tissue growth. Colostrum is a rich source of bioactive proteins,[1,2] which could account for its prescribed actions. Although colostrum might contain proteins with anabolic actions that have not yet been described, a possible mechanism of action could come from insulin-like growth factor-I (IGF-I). It is the most abundant and well-described growth factor in colostrum and has the same amino acid structure in bovines and humans.[3] Administration of IGF-I stimulated muscle protein synthesis in animals[3,4] and humans.[5] Colostrum supplementation may increase plasma IGF-I concentrations, which has a direct action on tissue growth, or IGF-I might increase intestinal maturation and, with that increase, nutrient uptake. However, Buckley et al.[6,7] found no increases in plasma IGF-I levels with the use of bovine colostrums (Intact™, GNC, Pittsburgh, PA, USA), whereas Mero et al.[8] found changes in IGF-I levels after training in subjects who had ingested bovine colostrums, although the magnitude of change was small and remained within the normal physiologic range. In addition to IGF-I, bovine colostrum contains growth hormone. Growth hormone also exerts a specific effect on metabolism.[9] In contrast to IGF-I, this form of bovine growth hormone does not bind to the human growth hormone receptor.[10]

Because colostrum contains components forbidden by the International Olympic Committee (IOC; e.g., IGF and growth hormone), whether to add colostrum to the IOC's list of banned substances is being debated. The purpose of this study was to investigate 1) whether colostrum supplementation (60 g/d) has any effect on plasma levels of IGF-I and its binding protein (insulin-like growth factor binding protein-3 [IGFBP3]) and 2) whether 4 wk of supplementation would have any effect on a regular urine drug test in an IOC-accredited laboratory.

MATERIALS AND METHODS

Subjects

Nine, healthy, well-trained, competitive male athletes participated in the study. Mean age was 25 y (range, 19–29), mean height was 1.83 m (range, 1.72–1.89 m), and mean body weight was 74.8 kg (range, 68.6–86.5 kg). Subjects were engaged in endurance training for at least 5 h/wk before the study. During the 4 wk of supplementation, their training programs did not change. The following exclusion criteria were used: intolerance to cow's milk protein, lactose intolerance, any medical condition requiring medication might affect the results of the study, or having taken ergogenic substances, nutritional supplements, or hormonal or chemical components within 6 wk before the start of the study.

Design

The study was in accordance with the Declaration of Helsinki and Dutch law and was approved by the local medical ethical committee. According to the guidelines, full written informed consent was signed by each subject.

Correspondence to: Harm Kuipers, MD, PhD, Department of Movement Sciences, PO Box 616, 6200 MD Maastricht, The Netherlands. E-mail: harm.kuipers@bw.unimaas.nl

Nutrition 18:566–567, 2002

0899-9007/02/$22.00
PII S0899-9007(02)00800-6

Name

Jonathan David BUCKLEY

Contact Details

16 New York Road
Aberfoyle Park
South Australia, 5159

Tel: [08] 8370 4991 (home)
Tel: [08] 8302 1853 (work)
Mobile: 0417 880030

Other

DOB: 4 December 1963
Married to Liz with 2 children (Thomas 3 y.o.) and William (1 y.o.)

Executive Summary

Appointments Co-Director, Australian Technology Network Centre for Metabolic Fitness; Deputy Director, Nutritional Physiology Research Centre, School of Health Sciences, University of South Australia; Associate Professor, School of Health Sciences, University of South Australia; Visiting Research Fellow, University of Adelaide.

Research profile Major research interests in nutrient and exercise effects on body composition (obesity), health, and physical performance. Invited speaker at major international (International Congress on Obesity Satellite) and national meetings (Australian Society for the Study of Obesity, Australian Dairy Conference, Sports Medicine Australia. Member Nutrition Society of Australia. Invited by Food Standards Australia and New Zealand (FSANZ) to review health benefits of omega-3 fatty acids to inform regarding pre-approval of health claims. Regular invitations to review grant applications (Australian Research Council, National Health and Medical Research Council, National Heart Foundation) and manuscripts for international scientific medical and exercise journals.

Research training and teaching Current principal supervisor of 2 PhD students and 1 Honours student, and joint supervisor of an additional 4 PhD students and 7 Honours students. Joint supervisor of 3 postdoctoral fellows, 1 research assistant, 2 technical assistants and 1 administrative assistant. Currently lecture in Physiology to Physiotherapy and Podiatry undergraduate students at University of South Australia. Won University wide teaching award in 2005 for high performance in teaching, and consistently achieve amongst the highest scores in student evaluations of teaching performance within the School of Health Sciences. Extensive experience in curriculum development.

Research grants Currently holding Australian Competitive Grants (NHMRC, ARC Linkage, NHF) totalling $3,611,453 and other grants totalling $2,886,700 (ATN Challenge and primarily industry funded grants). Previously held Australian Competitive Grants (Diabetes Australia) totalling $30,000, and other grants (Industry) totalling $1,094,737 (primarily industry funded grants). Total research funding past and present from all sources $7,622,890.

Research publications Research findings published in premier exercise, nutrition and physiology journals including Obesity (formerly Obesity Research) (IF 4.0), American Heart Journal (IF 3.6), Medicine and Science in Sports and Exercise (IF 2.8), and Nutrition (IF 2.1). Research findings also published in 17 confidential research reports for industry and/or government.

Research translation Contribution to changes in national elite athlete testing protocols. Contribution to national food policy development through FSANZ. Evaluation of health benefits of foods for the food industry leading to the introduction of new food products into the food supply. Development of intellectual property leading to the registration of patents for food products which improve health and athletic performance.

Academic Qualifications

1997	PhD	University of Adelaide, Adelaide, South Australia
1992	BSc (Hons)	University of Adelaide, Adelaide, South Australia
1991	BAppSc	University of South Australia, Adelaide, South Australia

Current Appointments

Associate Professor
School of Health Sciences
University of South Australia
Appointed in 2006

Co-Director
Australian Technology Network Centre for Metabolic Fitness
School of Health Sciences
University of South Australia
Appointed in 2005

Deputy Director
Nutritional Physiology Research Centre
School of Health Sciences
University of South Australia
Appointed in 2003

Visiting Research Fellow
School of Molecular and Biomedical Sciences
University of Adelaide
South Australia 5005
Appointed in 2001

Past Appointments

2002-05 *Senior Lecturer*
School of Health Sciences, University of South Australia

2002 *Acting Head of School (2 month)*
School of Physical Education, Exercise and Sport Studies
University of South Australia

1998-04 *Program Director, Bachelor of Applied Science (Honours)(Human Movement)*
School of Health Sciences, University of South Australia

1999-03 *Director, Centre for Research and Education in Sports Science*
School of Physical Education, Exercise and Sport Studies
University of South Australia

1999-01 *Lecturer (Level B)*
School of Physical Education, Exercise and Sport Studies
University of South Australia

1996-98 *Lecturer (Level A)*
School of Physical Education, Exercise and Sport Studies
University of South Australia

Professional Awards

1997 – 2006	*Supported Researcher Award for high performance in research* University of South Australia.
2005	*Best paper* Australian Society for Medical Research Conference, Adelaide.
2005	*Supported Teacher Award for high performance in undergraduate teaching* University of South Australia.
2004	*Best paper* American Oil Chemists Society (Australasian Chapter) Conference, Adelaide.
1994	*Barbara Crase Bursary for academic excellence* Australian Federation of University Women, University of Adelaide.
1993	*Astra Prize - Best student poster* Australian Physiological and Pharmacological Society, Adelaide.
1992 – 1995	*Australian Postgraduate Research Award* University of Adelaide, South Australia

Overview of Undergraduate Teaching

Current teaching

- Physiology (Physiotherapy and Podiatry programs)

Past teaching

- Human Anatomy and Physiology (Human Movement program)
- Exercise Biochemistry (Human Movement program)
- Exercise Physiology (Human Movement program)
- Curriculum development (BAppSc (Hons)(Human Movement))
- Curriculum development (Bachelor of Health Sciences (Honours))

Overview of Research Program

Athletic performance

- Amino acid supplements to improve athletic performance and recovery
- Protein hydrolysates for improving recovery from exercise
- Omega-3 fatty acid effects on exercise performance and recovery in elite athletes
- Improvement of elite athlete testing methodologies

Obesity and cardiovascular risk

- Efficacy and safety of low-carbohydrate diets for weight loss
- Effects of dietary weight loss and exercise on reproductive function in polycystic ovary syndrome
- Omega-3 fatty acid effects on obesity, cardiovascular risk and inflammation
- Effects of green tea polyphenols combined with exercise on obesity and cardiovascular risk
- Effects of cocoa polyphenols and exercise on body composition, cardiovascular risk and cognition
- Effect of green tea polyphenols on blood glucose control in glucose intolerance
- Effects of dietary weight loss and exercise on vascular function in type 2 diabetes

- Effects of dietary weight loss and resistance training on glycemic control in indigenous Australians
- Developing a cohort of indigenous health research leaders
- Efficacy of cognitive behavioural therapy for reducing obesity in adolescents
- Efficacy of a lifestyle education program for reducing obesity in a rural community
- Effect of soy and dairy protein on cardiovascular disease risk and cognitive function

Current Research Collaborations

Athletic performance

- Amino acid supplements, athletic performance and recovery (P Bourdon, SA Sports Institute)
- Protein hydrolysates and recovery (M DeNichilo, TGR BioSciences, Adelaide)
- Improving elite athlete testing (P Bourdon, S Woolford, SA Sports Institute)

Obesity and cardiovascular risk

- Low carbohydrate diets (P Clifton, M Noakes, G Brinkworth, CSIRO Human Nutrition, Adelaide)
- Polycystic ovary syndrome (P Clifton, M Noakes, G Brinkworth, CSIRO Human Nutrition, Adelaide)
- Omega-3 fatty acids (A Ferrante, Adelaide Women's and Children's Hospital)
- Vascular function in diabetes (P Clifton, M Noakes, G Brinkworth, CSIRO Human Nutrition, Adelaide)
- Cognitive behavioural therapy (J Walkley, RMIT University, Melbourne)
- Lifestyle education in rural communities (M Haas, University of Technology Sydney; G Misan Spencer Gulf Rural Health School, University of Adelaide)
- Soy and dairy proteins (T Mori, University of Western Australia; B Meyer, University of Wollongong)

Peer Review

Invited Reviewer - Manuscripts

- Journal of Applied Physiology (2005, 2006)
- European Journal of Applied Physiology (2004. 2005)
- Medicine and Science in Sports and Exercise (2002)
- International Journal of Sports Nutrition (2002)
- Journal of Science and Medicine in Sport (2003, 2004, 2005)
- Lipids (2005)
- Obesity Research and Clinical Practice (2006)

Invited Reviewer - Grants

National Health and Medical Research Council

- Project grants (2005, 2006)

Australian Research Council

- Discovery grants (2006)

National Heart Foundation

- Project grants (2005, 2006)

Thesis examination

Honours Theses (x12). General Examiner. School of Health Sciences, *University of South Australia*, 1999 - 2006

PhD Thesis. The influence of bovine colostrum supplementation on immune variables and exercise performance in trained cyclists. School of Human Movement Studies, *University of Queensland*, 2006.

Scientific Discipline Involvement

Membership of Professional Organisations

- Nutrition Society of Australia (since 2003)
- Australian Society for Medical Research (2000 - 2002)

Professional Contributions - Current

University of South Australia

- Member, Division of Health Sciences Research Management Committee, 2001 – ongoing
- Member, School of Health Sciences Research Management Committee, 2004 – ongoing
- Reviewer, Postgraduate student research proposals, School of Health Sciences, 2004 - ongoing

Professional Contributions - Previous

Sports Medicine Australia

- Reviewer, Abstracts for Annual Scientific Conference, Adelaide, 1998

University of South Australia

- Chair, School of Health Sciences Steering Committee for Rationalisation of Undergraduate Teaching , 2003 - 2004
- Student Counsellor, Human Movement Program, School of Physical Education Exercise and Sport Studies, 2001 – 2004
- Member, Curriculum Development Committee for Bachelor of Health Science (Honours) and Bachelor of Applied Science (Honours) (Specialisation), 2003
- Member, Steering Committee for Research Centre Development, Division of Health Sciences – 2003
- Member, Academic Board, 1996 - 1998
- Chair, Curriculum Development Committee for Bachelor of Applied Science (Honours)(Human Movement), 1997

Supervision

Current PhD students

- Mr Pitre Bourdon (Principal Supervisor). University of South Australia. Thesis title: Blood lactate transition thresholds in elite athletes.
- Ms Rebecca Thomson (Principal Supervisor). University of South Australia. Thesis title: Lifestyle strategies for the management of reproductive function in Polycystic Ovary Syndrome.
- Ms Alison Hill (co-supervisor). University of Adelaide. Thesis title: Effects of omega-3 fatty acids and exercise on body composition, cardiovascular risk and inflammation.

• Ms Alicia Thorp (co-supervisor). University of Adelaide. Thesis title: Effect of soy and dairy proteins on cardiovascular risk and cognitive function.

• Mr Kade Davison(co-supervisor). University of Adelaide. Thesis title: Cocoa polyphenols and exercise: effects on body composition, vascular function and cognition.

• Ms Tahna Pettman (co-supervisor). University of South Australia. Thesis title: A diet and exercise education program to reduce obesity and cardiovascular risk in a rural community.

Past PhD students

• Dr Grant Brinkworth (Principal Supervisor). University of South Australia. Thesis title: Physiological effects of oral bovine colostrum supplementation. Completed 2003.

Past Masters students

• Ms Marion Abbott (Principal Supervisor). University of South Australia. Thesis title: The effect of an oral bovine colostrum supplement on physical work capacity and body composition. Completed 2002.

Current Honours students

• Mr Ian Pateyjohns (Principal Supervisor). University of South Australia. Thesis title: Comparison of three bioelectrical impedance methods with DEXA in overweight and obese males.

• Ms Margarita Tsiros (co-supervisor). University of South Australia. Thesis title: Cognitive behavioural therapy to treat overweight and obesity in adolescents.

• Mr Stelios Sioutis (co-supervisor). University of Adelaide. Thesis title: Regular consumption of omega-3 enriched pork increases docosahexaenoic acid incorporation into erythrocyte membranes.

• Ms Catherine Milte (co-supervisor). University of Adelaide. Thesis title: DHA intake affects biomarkers of omega-3 status and cardiovascular risk.

• Mr Thomas Wycherley (co-supervisor). University of Adelaide. Thesis title: Weight loss with and without aerobic exercise improves cardiovascular disease risk factors but not flow-mediated dilatation in obese subjects with type 2 diabetes.

• Ms Angela Halyburton (co-supervisor). University of Adelaide. Thesis title: Effects of a low-carbohydrate diet on mood and cognitive function.

• Ms Rosali Kenyon (co-supervisor). University of Adelaide. Thesis title: Effects of a low-carbohydrate diet on markers of DNA damage.

• Ms Anna Puckridge (co-supervisor). University of Adelaide. Thesis title: Effects of a low-carbohydrate diet on physical function and exercise capacity.

Past Honours students

• Ms Rebecca Thomson (Principal Supervisor). University of South Australia. Thesis title: Effectiveness of emu oil in reducing exercise-induced muscle soreness and inflammation. Completed 2005. *Awarded First Class Honours.*

• Mr Shane Burgess (Principal Supervisor). University of South Australia. Thesis title: The effect of omega-3 PUFA on exercise performance, recovery, inflammatory markers and cardiovascular risk factors in professional Australian Rules football players. Completed 2004. *Awarded First Class Honours.*

• Mr Stuart Graham (Principal Supervisor). University of South Australia. Thesis title: Development of a simple method for determining the state of recovery in young athletes. Completed 2004. *Awarded First Class Honours.*

• Mr Andrew Beck (Principal Supervisor). University of South Australia. Thesis title: The evaluation of a new method for analysis of treadmill running data. Completed 2004. *Awarded 2A Honours.*

• Ms Alicia Thorp (Co-supervisor). University of South Australia. Thesis title: Digital volume pulse oximetry: a reliable assessor of endothelial function and arterial compliance? Completed 2003. *Awarded First Class Honours.*

• Ms Kelly Leah (Co-supervisor). University of South Australia. Thesis title: The effect of short term exposure to increased carbon dioxide levels on choice response time, pulmonary ventilation and heart rate in normal healthy individuals. Completed 2001. *Awarded First Class Honours.*

• Ms Susan Thomas (Co-supervisor). University of South Australia. Thesis title: The effect of short term exposure to raised levels of CO_2 on motor performance and physiological responses in normal individuals. Completed 2001. *Awarded First Class Honours.*

• Mr Simon Stolcman (Principal Supervisor). University of South Australia. Thesis title: Bovine colostrum and URTI infection in young children. Completed 2001. *Awarded 2A Honours.*

• Mr Grant Brinkworth (Principal Supervisor). University of South Australia. Thesis title: Effects of oral bovine colostrum supplementation in elite rowers. Completed 1999. *Awarded First Class Honours.*

Research Staff Supervision – current

Postdoctoral Research Fellow, Nutritional Physiology Research Centre (shared supervision). Dr Barbara Parker, PhD, 2006 – ongoing

Postdoctoral Research Fellow, Nutritional Physiology Research Centre (shared supervision). Dr Narelle Berry, PhD, 2006 – ongoing

Postdoctoral Research Fellow, Nutritional Physiology Research Centre (shared supervision). Dr Natalie Sinn, PhD, 2006 – ongoing

Research Assistant, Nutritional Physiology Research Centre (shared supervision). Ms Katie Boyd, 2006 - ongoing

Technical Assistant, Nutritional Physiology Research Centre (shared supervision). Ms Amanda Jager, 2006 – ongoing

Technical Assistant, Nutritional Physiology Research Centre (shared supervision). Ms Keren Kneebone, 2006 - ongoing

Administrative Assistant, ATN Centre for Metabolic Fitness (shared supervision). Ms Erin Riley, 2005 - ongoing

Research Staff Supervision - past

Postdoctoral Research Fellow, Nutritional Physiology Research Group, School of Health Sciences University of South Australia (shared supervision). Dr Karen Murphy investigated the effects of omega-3 fatty acids and exercise on body composition and exercise performance and recovery. Her studies are currently being written up for publication. She moved to a group at the University of Adelaide after being with our group for 18 months.

Postdoctoral Research Fellow, Nutritional Physiology Research Centre, School of Health Sciences University of South Australia (shared supervision). Dr Alison Coates worked on a number of projects examining health benefits of nutrients. Some of her work is published and other studies are currently being written up for publication. She took up an academic position with the University of South Australia after working with our group for 2 years and continues to collaborate.

Teaching

- Physiology 100 (2006 – ongoing), 39 x 1.0 hr lectures, 130 – 140 students
- Physiology 101 (2006 - ongoing), 39 x 1.0 hr lectures, 130 – 140 students
- Physiology 100 (2004 – 2005), Course Coordination, 39 x 1.0 hr lectures, 130 – 140 students
- Physiology 101 (2004 – 2005), Course Coordination, 39 x 1.0 hr lectures, 130 – 140 students
- Human Anatomy and Physiology 1 (1997 - 2005), Course Coordination, 26 x 1.0 hr lectures, 3 x 2 hr practicals, 120 - 130 students

- Human Anatomy and Physiology 2 (1997 - 2005), Course Coordination, 26 x 1.0 hr lectures, 3 x 2 hr practicals, 120 - 130 students
- Exercise Biochemistry (1996 - 2005), Course Coordination, 26 x 1.0 hr lectures, 3 x 2 hr practicals, 30 - 40 students
- Exercise Physiology (1996 - 1997), Course Coordination, 26 x 1.0 hr lectures, 3 x 2 hr practicals, 60 - 70 students
- Curriculum development (BAppSc (Hons)(Human Movement)), 1997
- Curriculum development (Bachelor of Health Sciences (Honours)), 2003

Involvement in the Wider Community

South Australian Sports Institute

- Fitness Advisor, Athletics (Mr Nik Hagikostas coach), 2004
- Fitness Advisor, Weightlifting (Mr Rick Crump coach), 1990 – 1991

South Australian National Football League

- Fitness Advisor, Norwood Football Club (Mr Neil Craig coach), 1990 - 1991

Australian Society for Medical Research

- Careers in Science presentation for High School Students, 2001

Australian Olympic Committee

- Host, Drugs in Sport Initiative Presentation to junior athletes, 2000

Research Profile

International – invited speaker

2006 International Congress on Obesity Physical Activity and Obesity Satellite, Brisbane, 2006

- *"Simpler diet and exercise solutions for managing obesity"*

International – session chair

2006 International Congress on Obesity Physical Activity and Obesity Satellite

- Chair, Diet and Exercise Session, Brisbane, 2006

National – invited speaker

2005 Australian Society for the Study of Obesity, Glenelg
"Engaging the food industry into action on obesity".

2004 Australian Dairy Conference, Shepparton.
Member of expert panel on health benefits of dairy foods

2000 Dairy Research Foundation, Kensington.
"Does a diet of colostrum improve athletic performance?"

1999 Professional and Amateur National Coaching Society (PANCS), Sydney.
"Effects of bovine colostrum on athletic performance"

National – session chair

2005 Nutrition Society of Australia, Annual Scientific Meeting, Melbourne.
Chair, Plenary Session on Nutrient – Gene interactions

2000 Sports Medicine Australia, Annual Scientific Meeting, Brisbane.
Chair, Conference Session on Exercise Physiology

1998 Sports Medicine Australia, Annual Scientific Meeting, Adelaide.
Chair, Conference Session on Exercise Immunology and Endocrinology

Local – invited speaker

2005 Healthy Development Adelaide, University of Adelaide.
"Obesity and lifestyle"

2004 Sports Medicine Australia (SA Branch), Kidman Park.
"Exercise physiology for physicians"

2001 Sports Medicine Australia (SA Branch), Glenelg.
"Bovine colostrum improves vertical jump performance"

2001 Defence Science and Technology Organisation, Salisbury.
"Performance effects of bovine colostrum"

2000 Australian Institute of Sport, Canberra.
"Effects of bovine colostrum on athletic performance"

1999 Department of Obstetrics and Gynaecology, University of Adelaide.
"Effects of bovine colostrum on athletic performance and IGF-1"

Community presentations

2006 University of South Australia Body of Knowledge Public Lecture Series, Adelaide.
"Simpler diet and exercise solutions for managing obesity"

2006 University of South Australia Blue Sky Public Lecture Series, Whyalla.
"Simpler diet and exercise solutions for managing obesity"

Research Grant Support

Research Grants - Current

Title	Investigators	Funding source	Year	Amount
Building a cohort of indigenous research leaders in community health development	McDermott R Esterman A Buckley J d'Abbs P Tsey K Segal L	NHMRC Capacity Building Grant 456402	2007 - 2011	$2,376,107
Lifestyle strategies for the	Clifton P Noakes M Buckley J	NHMRC Project Grant 401817	2006 - 2007	$375,500

management of reproductive function in polycystic ovary syndrome	Brinkworth G			
Long-term efficacy and safety of low-carbohydrate diets	Clifton P Noakes M Brinkworth G Buckley J Fenech M Wilson C	NHMRC Project Grant 401818	2006 - 2008	$488,750
Effect of Vespa Amino Acid Mixture (VAAM) on fat oxidation during exercise, and exercise performance, in well-trained athletes	Buckley J Howe P Coates A Parker B Abarno D	Meiji Dairies Corporation, Japan	2006 - 2007	$243,500
Effect of a novel whey protein hydrolysate on inflammation and recovery of muscular function following muscle damage induced by eccentric exercise	Buckley J Howe PRC	MG Nutritionals, Brunswick, Vic	2006	$80,200
Effect of dairy based replacement meals on mood and appetite regulation in normal weight and obese subjects	Howe PRC Buckley J Parker B	MG Nutritionals, Brunswick, Vic	2006	$80,000
Effects of supplementing the diet with cocoa or omega-3 fatty acids, alone or in combination, on blood pressure and body composition	Howe PRC Buckley J Morris AM	Food Innovation Grant – National Food Industy Strategy and Effem Foods Pty Ltd (Masterfoods)	2006 - 2007	$368,000
The relationship between omega-3 fatty acid intake and risk of cardiovascular disease. A review of a diet-disease relationship.	Howe PRC Mori T Buckley J	Food Standards Australia and New Zealand	2006	$12,000
Evaluation of a green tea extract on blood glucose regulation	Howe PRC Buckley JD Coates AM	DSM Nutritional Products	2006	$25,000

ATN Centre for Metabolic Fitness: diet and lifestyle strategies to optimise health and reduce the burden of obesity-related disease	Howe PRC McDermott R Buckley JD et al.	ATN Challenge. ATN University Network.	2005 - 2009	$1,985,000
Development and application of an index for substantiating health benefits of omega-3 enriched foods.	Howe PRC Buckley JD Ferrante A	ARC Linkage Grant LP0561211 (Industry partners: Bartlett Grain and Australian Pork Ltd)	2005 - 2006	$251,096
The effects of a very-low carbohydrate, weight reduction diet compared to a low saturated fat diet on physical capacity, mood and cardiovascular disease risk in subjects with metabolic syndrome	Clifton P Noakes M Buckley J Brinkworth G	National Heart Foundation Grant-in-aid.	2005 - 2006	$120,000

Research Grants - Past

Title	Investigators	Funding source	Year	Amount
Effect of energy restriction and exercise training on vascular endothelial function and oxidative stress in obese subjects with type 2 diabetes	Clifton PM Noakes M Brinkworth GD Buckley JD McArthur R Sullivan C	Diabetes Australia Research Trust	2005	$30,000
Effects of a novel nutrient on abdominal fat mass in overweight/obese volunteers.	Howe PRC, Buckley JD, Morris A, Hill A, Ninio D, Taylor J	DSM Nutritional Products	2005	$215,000
Anti-inflammatory effects of active emu oil.	Buckley J, Howe P, Morris A.	Technology Investments Corporation Pty Ltd.	2005	$49,946

Impact of dietary omega-3 fatty acid supplementation on asymmetric dimethylarginine (ADMA), a novel cardiovascular risk factor.	Morris A Howe P Buckley J Sawyer T	University of South Australia Competitive Grant	2005	$15,000
Impact of cocoa polyphenols and exercise on abdominal adiposity.	Howe PRC Buckley JD Morris A	Effem Foods Pty Ltd (Masterfoods).	2005	$105,000
Assessment of the glycemic index of foods containing waxy barley	Howe PRC Buckley JD Morris A Thorp A	Grain Research Development Corporation.	2005	$20,000
Development and application of an index for substantiating health benefits of omega-3 enriched foods.	Howe P Buckley J Ferrante A	University of South Australia, Division of Health Sciences National Competitive Grant Top-up Grant.	2005	$8,000
Impact of Vinlife® grape seed polyphenols in combination with aerobic exercise on cardiovascular and metabolic fitness.	Morris A Buckley J Howe P	University of South Australia ARC Linkage Project Development Incentive Grant.	2005	$2,500
Effect of whey protein supplements on blood pressure	Howe P Buckley J Morris A	Effem Foods Pty Ltd (Masterfoods).	2005	$35,000
Effect of a novel food ingredient on the glycemic index of food	Howe PRC Buckley JD Murphy KJ	Wacker-Chemie GmbH.	2004	$22,000
Development of the Nutritional Physiology Research Facility Infrastructure	Howe PRC Buckley JD	University of South Australia Research Infrastructure Grant	2004	$22,000
Nutritional Physiology Research Group Postdoctoral Fellowship Grant	Howe PRC Buckley JD	University of South Australia, Division of Health Sciences	2004 - 2005	$60,000

Determination of vascular dilatation during exercise using the PulseTrace Digital Volume Pulse Plethysmograph	Buckley JD Howe PRC	University of South Australia ARC Linkage Project Incentive Scheme	2004	$2,500
Effects of green tea polyphenols on body composition and cardiovascular disease risk	Buckley JD Howe PRC	University of South Australia ARC Linkage Project Incentive Scheme	2004	$5,000
The effect of a nutritional supplement on athletic performance in Australian Rules footballers	Howe PRC Buckley JD Murphy KJ	Port Adelaide Football Club Inc	2003	$25,000
Effect of omega-3 polyunsaturated fatty acids and regular exercise training on cardiovascular risk factors in subjects with metabolic syndrome	Howe PRC Buckley JD Murphy KJ	University of South Australia Strategic Initiatives and Research Development Grant	2003	$56,000
Effect of omega-3 fatty acids on body composition in obesity	Howe PRC Saint DA Buckley JD	University of Adelaide B3 Establishment grant.	2003	$13,800
Digital video analysis system	Buckley JD	University of South Australia, Division of Health Sciences Research Infrastructure Grant	2002	$11,000
COSMED portable indirect calorimeter	Buckley JD	University of South Australia, Division of Health Sciences Research Infrastructure Grant	2001	$15,000
Effect of oral bovine colostrum supplementation on acute and short-term changes in plasma insulin-like growth factor I concentration and intestinal nutrient absorption	Buckley JD	Numico Research Australia Pty Ltd	2001	$4,000

Effect of oral bovine colostrum supplementation on muscular strength and hypertrophy	Buckley JD	Numico Research Australia Pty Ltd	2000	$67,000
Effect of oral bovine colostrum supplementation on rowing performance in elite rowers	Buckley JD	Numico Research Australia Pty Ltd	2000	$15,000
Quinton treadmill	Buckley JD	University of South Australia, Division of Health Sciences Research Infrastructure Grant	2000	$15,000
Effect of oral bovine colostrum supplementation on exercise performance and recovery in elite rowers	Buckley JD	Numico Research Australia Pty Ltd	1999	$25,000
Small intestine permeability and inflammation in athletes, effects of probiotics	Butler RG Buckley JD	Nestle Australia Pty Ltd	1999	$42,808
Indirect calorimetry system	Buckley JD	University of South Australia, Division of Health Sciences Research Infrastructure Grant	1999	$10,000
Effect of oral bovine colostrum supplementation on body compostion and endurance running performance	Buckley JD	Numico Research Australia Pty Ltd	1998	$100,000
Effect of oral bovine colostrum supplementation on body compostion, strength and power	Buckley JD	Numico Research Australia Pty Ltd	1998	$96,000
Efficacy of a topical ketoprofen (NSAID) cream	Buckley JD	Soltec Pty Ltd (on behalf of Pfizer Pty Ltd)	1998	$8,700
Efficacy of a topical piroxicam (NSAID) cream	Buckley JD	Soltec Pty Ltd (on behalf of Pfizer Pty Ltd)	1998	$15,000

Effect of oral bovine colostrum supplementation on body composition and physical work capacity	Buckley JD	Numico Research Australia Pty Ltd	1997	$51,483
Development of an exercise biochemistry and nutrition research laboratory	Buckley JD	University of South Australia, Faculty of Education Strategic Initiatives Grant	1996	$50,000

Publications

Refereed Journal Articles

1. Pateyjohns IR, Brinkworth GD, Buckley JD, Noakes M, Clifton PM. Comparison of three bioelectrical impedance methods with DEXA in overweight and obese males. Obesity (in press) 2006.

2. Hill AM, LaForgia J, Coates AM, Buckley JD, Howe PRC. Estimating abdominal adipose tissue with dual energy x-ray absorptiometry and anthropometry. Obesity (in press) 2006.

3. Brinkworth GD, Noakes M, Buckley JD, Clifton PM. Weight loss improves heart rate recovery in overweight and obese men with features of metabolic syndrome. American Heart Journal 152:693.e1-693.e6, 2006.

4. Buckley, JD., Thorp AJ, Murphy KJ, and PRC Howe. Dose-dependent inhibition of the post-prandial glycaemic response to a standard carbohydrate meal following incorporation of a-cyclodextrin. Annals of Nutrition and Metabolism 50:108-114, 2006.

5. Brinkworth, GD., Buckley, JD. Slavotinek, JP., and Kurmis A. Effect of bovine colostrum supplementation on the composition of resistance trained and untrained limbs in healthy young men. European Journal of Applied Physiology, 91:53-60, 2004.

6. Brinkworth, GD. & Buckley, JD. Bovine colostrum supplementation does not affect plasma buffer capacity or haemoglobin content in elite female rowers. European Journal of Applied Physiology, 91:353-356, 2004.

7. Brinkworth, GD. & Buckley, JD. Bovine colostrum supplementation does not affect nutrient absorptive capacity in healthy young men. Nutrition Research, 23(12):1619-1629, 2003.

8. Buckley, JD., Bourdon, PC., and SM Woolford. Effect of measuring blood lactate concentrations using different automated lactate analysers on blood lactate transition thresholds. Journal of Science and Medicine in Sport. 6(4): 408-421, 2003.

9. Buckley, JD,. GD. Brinkworth and MJ. Abbott. Effect of bovine colostrum on anaerobic exercise performance and plasma IGF-I. Journal of Sports Sciences, 21: 577-588, 2003.

10. Brinkworth, GD. & Buckley, JD. Concentrated bovine colostrum protein supplementation reduces the incidence of self-reported symptoms of upper respiratory infection in adult males. European Journal of Nutrition 42(4): 228-232,2003.

11. Brinkworth, GD, JD Buckley, PC Bourdon, JP Gulbin and AZ David. Oral bovine colostrum supplementation enhances buffer capacity but not rowing performance in elite female rowers. International Journal of Sports Nutrition, 12: 349-363, 2002

12. Buckley J.D., M.J. Abbott, G.D. Brinkworth, and P.B.D. Whyte. Bovine colostrum supplementation during endurance running training improves recovery, but not performance. Journal of Science and Medicine in Sport. 5(2): 65-79, 2002.

13. Sargent C., Scroop G.C., Nemeth P.M., Burnet R.B. and Buckley J.D. Maximal oxygen uptake and lactate metabolism are normal in chronic fatigue syndrome. Medicine and Science in Sports and Exercise 34(1):51-56, 2002.

14. Buckley J.D., Scroop, G.C. and Catcheside, P.G. No difference in net uptake or disposal of lactate by trained and untrained forearms during incremental lactate infusion. European Journal of Applied Physiology. 85(5):412-419, 2001

15. Buckley J and A Scammell. Does a diet of colostrum improve athletic performance? Asian-Australasian Journal of Animal Sciences 13(Suppl.): 312-315, 2000.

16. Gore, C.J., S.C. Little, A.G. Hahn, G.C. Scroop, K.I. Norton, P.C. Bourdon, S.M. Woolford, J.D. Buckley, T. Stanef, D.P. Campbell, D.B. Watson, D.L. Emonson. Reduced performance of male and female athletes at 580 m altitude. European Journal of Applied Physiology 75:136-143, 1997.

17. Buckley J.D., G.C. Scroop and P.G. Catcheside. Lactate disposal in resting trained and untrained forearm skeletal muscle during high intensity leg exercise. European Journal of Applied Physiology 67(4):360-366, 1993.

Book Chapters

18. Buckley J, Hill A, Coates A, Howe P. Simpler diet and exercise solutions for managing obesity. In: Physical Activity and Obesity, Hills AP, Bryne NM and King NA (eds), Smith-Gordon and Co, London. 2006; pp 100-104.

Editorials

19. Buckley, JD. Bovine Colostrum: does it improve athletic performance? Nutrition 18(9): 776-777, 2002.

Conference papers (Full refereed papers)

20. Buckley J and A Scammell. Does a diet of colostrum improve athletic performance? Current topics in dairy production 5:91-94, 2000.

21. Scroop, G.C. and J.D. Buckley. Lactate metabolism during exercise. Invited Review. Proceedings of the Australian Physiological and Pharmacological Society 24 (2):81-86, 1993.

Conference abstracts (Refereed)

22. PRC Howe, AM Hill, AM Coates, JD Buckley, R Ross, F Thielecke. Can EGCG reduce abdominal fat in obese subjects? 47th Annual meeting of the American College of Nutrition, Nevada, October, 2006.

23. GD Brinkworth, M Noakes, JD Buckley, PM Clifton. Weight loss improves heart rate recovery in overweight and obese men with features of metabolic syndrome. Obesity Reviews 7(2 Suppl):306, 2006.

24. Howe P, A Coates, A Hill, J Buckley, J Taylor, R Ross, F Thielecke. Cardiovascular and metabolic effects of epigallocatechin gallate (EGCG) supplementation in conjunction with an exercise program in overweight women. Obesity Reviews (7(2 Suppl):310, 2006.

25. GD Brinkworth, M Noakes, JD Buckley, PM Clifton. Weight loss improves heart rate recovery in overweight and obese men with features of metabolic syndrome. Physical Activity and Obesity, Satellite conference of the 10th International Conference on Obesity, Brisbane, September 2006.

26. J Buckley. Simpler diet and exercise strategies for managing obesity. Physical Activity and Obesity, Satellite conference of the 10th International Conference on Obesity, Brisbane, September 2006.

27. AM Coates, JD Buckley, A Ferrante, PRC Howe. Dose-dependent effects of DHA-rich tuna oil on cardiovascular and inflammatory markers. 7th Congress of the International Society for the Study of Fatty Acids and Lipids, Cairns, July, 2006.

28. AM Hill1, JD Buckley, KJ Murphy, PRC Howe. Running on fish oil: benefits of omega-3 supplementation and exercise. 7th Congress of the International Society for the Study of Fatty Acids and Lipids, Cairns, July, 2006.

29. T Pettman, G Misan, K Warren, M Haas, K Owen, J Buckley, A Coates, P Howe. Countering metabolic syndrome through sustainable dietary and physical activity modification. Physical Activity and Obesity, Satellite conference of the 10th International Conference on Obesity, Brisbane, September 2006.

30. A Hill, J LaForgia, A Coates, J Buckley, PRC Howe. DXA and anthropometry to predict intra abdominal adipose tissue. Physical Activity and Obesity, Satellite conference of the 10th International Conference on Obesity, Brisbane, September 2006.

31. PC Bourdon, J Buckley. A single test for assessing physiological and performance parameters in rowers. 11th annual congress of the European College of Sports Sciences. Lausanne, July 2006.

32. A Thorp, A Coates, J Buckley, T Mori , J Hodgson, B Meyer, P Howe. Lack of effect of soy diets on LDL cholesterol. Australian Society for Medical Research, Adelaide, June 2006.

33. Buckley JD, Thomson R, Coates AM, Howe PRC. A novel hydrolysate of whey protein isolate enhances isometric muscle torque following eccentric exercise. Journal of the International Society of Sports Nutrition. 3 (1) S2, 2006.

34. JD Buckley, S Burgess, KJ Murphy, PRC Howe. Effects of omega-3 polyunsaturated fatty acids on cardiovascular risk, exercise performance and recovery in Australian Football League (AFL) players. Asia Pac J Clin Nutr 14:S57, 2005.

35. AM Hill, JD Buckley, KJ Murphy, DA Saint, AM Morris and PRC Howe. Combined effects of omega-3 supplementation and regular exercise on body composition and cardiovascular risk factors. Asia Pac J Clin Nutr 14:S57, 2005.

36. J Buckley, A Thorp, K Murphy, P Howe. Dose-dependent inhibition of the post-prandial glycemic response to a standard carbohydrate meal following incorporation of -cyclodextrin. Asia Pac J Clin Nutr 14:S77, 2005.

37. A Thorp, A Morris, J Buckley, T Mori , J Hodgson, B Meyer & P Howe. Isoflavones influence the extent of plasma cholesterol reduction by soy protein. Asia Pac J Clin Nutr 14:S75, 2005.

38. GD Brinkworth, IR Pateyjohns, JD Buckley. Evaluation of three bioelectrical impedance analysers to assess body composition in overweight and obese males. Asia Pac J Clin Nutr 14:S115, 2005.

39. Buckley J, Davison K, Bircher S, Hill A, Howe P. Obesity and cardiorespiratory fitness may differentially affect arterial function in sedentary subjects. Australian Society for the Study of Obesity, Adelaide, October 2005.

40. Hill A, Buckley J, Murphy K, Saint D, Howe P. Omega - 3 supplementation enhances body fat loss during exercise. 2nd International Symposium on Triglycerides and HDL, New York, July 2005

41. Thorp A, Morris A, Buckley J, Mori T, Hodgson J, Meyer B & Howe P. Lipid lowering effects of foods containing soy protein and isoflavones. Australian Society for Medical Research, Adelaide, June 2005. ***First Prize for Best Paper.***

42. Davison K, Bircher S, Hill A, Howe P, Buckley J. Improved cardiorespiratory fitness counteracts endothelial dysfunction in the obese. Australian Society for Medical Research, Adelaide, June 2005.

43. Ninio DM, AM Hill, JH Smith, K Murphy, J Buckley, DA Saint, PRC Howe. Fish oil enhances heart rate variability in overweight adults. American Oil Chemists Society (Australasian Chapter) Conference, Adelaide Dec 2004

44. Hill A, Buckley J, Murphy K, Saint D, Howe P. Cardiovascular & metabolic benefits following omega-3 fatty acid supplementation and exercise. American Oil Chemists Society (Australasian Chapter) Conference, Adelaide Dec 2004. ***First Prize for Best Paper.***

45. Worthley C, Hill A, Murphy K, Buckley J, Ferrante A, Howe P. Combined effects of omega-3 and exercise on leukocyte functions in subjects with cardiovascular risk factors. AOCS (Australasian Chapter) Conference, Adelaide Dec 2004

46. Hill AM, KJ Murphy, DA Saint, JD Buckley & PRC Howe. Combined benefits of Omega-3 and exercise for cardiovascular health. Australian Atherosclerosis Society and International Society for Vascular Biology, Barossa Valley, September, 2004.

47. Buckley JD, GD Brinkworth, E Southcott, RN Butler. Bovine colostrum and whey protein supplementation during running training increase intenstinal permeability. Asia Pacific Journal of Clinical Nutrition 13:s81, 2004.

48. Ninio DM, AM Hill, JH Smith, K Murphy, J Buckley, DA Saint, PRC Howe. Fish oil enhances heart rate variability in overweight adults. Cardiac Soc ANZ, Brisbane. Heart, Lung Circ 2004;13:S82

49. Thorp A, P Howe, K Murphy, J Buckley. Non-invasive assessment of arterial function using digital volume pulse oximetry. Australian Society for Medical Research, Adelaide, June 5-12, 2004.

50. Davison K, A Hill, A Thorp, C Worthley, K Murphy, P Howe, J Buckley. Digital volume pulse (DVP) analysis of vasodilator function during exercise. Australian Society for Medical Research, Adelaide, June 5-12, 2004.

51. Hill AM, KJ Murphy, DA Saint, JD Buckley & PRC Howe. Combined effects of omega-3 and moderate exercise on body fat and cardiovascular risk factors. Australian Society for Medical Research, Adelaide, June 5-12, 2004.

52. Hill, AM., Murphy, KJ., Saint, D., Buckley, J., and PRC Howe. Effect of omega-3 fatty acid supplementation and moderate-intensity exercise on body composition Nutrition Society, Dublin, 5-8 July 2004.

53. Buckley, JD and GD Brinkworth. Concentrated bovine colostrum protein supplementation reduces the incidence of self-reported symptoms of upper respiratory tract infection in adult males. Proceedings of the 32nd Annual Scientific Meeting of the Australasian Society for Immunology, Brisbane, Australia, Dec 8-12, pg 133, 2002.

54. Brinkworth, G.D., Buckley, J.D., Slavotinek, J.P., and Kurmis, A.P. Effect of bovine colostrum supplementation (intact™) on the composition of resistance trained and untrained limbs in healthy young men. Journal of Science and Medicine in Sport 5(4 Suppl.): 90, 2002.

55. Buckley, J., Bourdon, P.C., and Woolford, SM. Effect of different automated lactate analysers on the determination of blood lactate transition thresholds. Journal of Science and Medicine in Sport 5(4 Suppl.): 49, 2002.

56. Leahy, K., Thomas, S., Warden-Flood, A., Buckley, J., Binhendi, I. Effect of carbon dioxide on motor and physiological responses. Australian Journal of Physiotherapy 47: 271-272, 2001.

57. Buckley, JD, G.D. Brinkworth, J.P Slavotinek. Effect of bovine colostrum supplementation on the composition of resistance trained and non-trained limbs. Medicine and Science in Sports and Exercise 33(5):S340, 2001.

58. Buckley, J. P. Catcheside and G Scroop. Similar net fractional lactate extraction by endurance trained and untrained skeletal muscle during sodium lactate infusion. Proceedings of the 5th Annual congress of the European College of Sports Sciences. Jyvaskyla, Finland. July 2000, p182.

59. Buckley, J. Oral supplementation with concentrated bovine colostrum protein powder (intact™) improves vertical jump performance. Proceedings of the SA State conference of Sports Medicine Australia. Adelaide. March, 2000.

60. Buckley, J.D, M.J. Abbott, G.D. Brinkworth, P.B.D. Whyte. Bovine colostrum supplementation during training increases vertical jump performance. Proceedings of the International Congress on Sport Science Sports Medicine and Physical Education. Brisbane, Australia, September, 2000 p274.

61. Buckley, J.D., G.D. Brinkworth, P.C. Bourdon, J. Gulbin, E.J. Stilwell, A. David, N. Myers. Oral supplementation with bovine colostrum (intact™) improves rowing performance in elite female rowers. Proceedings of the 5th IOC World Congress of Sports Sciences, Sydney, 1999.

62. Sargent, C., P.M.D. Nemeth, G.C. Scroop, R.B. Burnett, J.D. Buckley. Normal exercise capacity in chronic fatigue syndrome. Proceedings of the European Conference on Chronic Fatigue Syndrome, Brussels, 1999.

63. Buckley, J. Colostrum - a performance enhancing substance. Proceedings of the 14th Annual conference of the Dairy Industry Association of Australia (New South Wales Division). Wentworth hotel, Sydney, 24-25 May, 1999.

64. Buckley, J., M. Abbott, G. Brinkworth, P. Whyte. Oral supplementation with bovine colostrum (intact™) increases vertical jump performance. Proceedings of the 4th Annual congress of the European College of Sports Science. Rome University of Motor Sciences. July 1999, p658.

65. Buckley, J., M. Abbott, S. Martin, G. Brinkworth, P. Whyte. Effect of an oral bovine colostrum supplement (intact™) on running performance. Proceedings of the Australian Conference of Science and Medicine in Sport. October 1998, p79.

66. Scroop, G., J. Buckley, R. Burnet, P. Nemeth, C. Sargent. Chronic fatigue syndrome and the female athlete. Proceedings of the Australian Conference of Science and Medicine in Sport. October 1998, p45.

67. Southcott, E., J. Buckley, R. Butler, S. Deakin, M. Abbott, S. Martin, D. Moore, G. Davidson. Changes in small intestinal permeability in athletes. Journal of Gastroenterology and Hepatology. 13:A124, 1998.

68. Southcott, E., J. Buckley, R. Butler, S. Deakin, M. Abbott, S. Martin, D. Moore, G. Davidson. Small intestinal permeability in sports athletes and IBD patients. Proceedings of the Australian Society for Medical Research. June 1998.

69. Buckley, J.D. and G.C. Scroop. Lactate kinetics in the resting forearm during incremental leg exercise. Proceedings of the Australian Physiological and Pharmacological Society 24 (2):186P, 1993. ***First Prize for Best Paper.***

Research reports to industry and/ or government

70. The effect of an oral bovine colostrum supplement (intact™) on body composition and physical work capacity during endurance exercise training. Report prepared for NorthField Laboratories, 1998.

71. The effect of an oral bovine colostrum supplement (intact™) on body composition and physical work capacity during strength and power training. Report prepared for NorthField Laboratories, 1998.

72. The efficacy of Liquipatch™ ketoprofen in an inflammatory model: delayed onset muscle soreness. Report prepared for Faulding Australia, 1998.

73. The efficacy of Liquipatch™ piroxicam in an inflammatory model: delayed onset muscle soreness. Report prepared for Faulding Australia, 1998.

74. The effect of an oral bovine colostrum supplement (Gastrogard-R™) on exercise performance and recovery in elite rowers. Report prepared for NorthField Laboratories, 1999.

75. The effect of an oral bovine colostrum supplement (intact™) on rowing performance in elite rowers. Report prepared for NorthField Laboratories, 2000.

76. Small intestinal permeability and inflammation in athletes – the effects of probiotics: running component. Report prepared for Nestle Australia and Adelaide Women's and Children's Hospital, 2000.

77. The effect of an oral bovine colostrum supplement (intact™) on the development of muscular strength and hypertrophy. Report prepared for NorthField Laboratories, 2000.

78. Effect of oral bovine colostrum supplementation (intact™) on acute and short-term changes in plasma insulin-like growth factor I concentration. Report prepared for Numico Research (Australia) Pty Ltd, 2001.

79. Effect of oral bovine colostrum supplementation (intact™) on plasma nutrient and insulin concentrations after standardised alanine and glucose feeding. Report prepared for Numico Research (Australia) Pty Ltd, 2001.

80. The effect of a novel food ingredient (confidential) on the glycaemic index of food. Report prepared for Wacker-Chemie GmbH, 2004.

81. Effects of omega-3 fatty acids on endurance exercise performance, recovery and markers of cardiovascular health in professional Australian rules footballers. Report prepared for the Port Adelaide Football Club Inc, 2004.

82. Pilot study to investigate the effects of the green tea extract TEAVIGO™, high in EGCG, on abdominal fat mass in overweight/obese volunteers. Report prepared for DSM Nutritional Products, 2005.

83. Effectiveness of emu oil in reducing exercise-induced muscle soreness and inflammation. Report prepared for Technology Investments Corporation, 2005.

84. Effects of supplementing the diet with whey protein, alone or combined with cocoa, on ambulatory blood pressure. Report prepared for Masterfoods Australia and New Zealand, 2006.

85. Howe PRC, Mori T and J Buckley. The relationship between omega-3 fatty acid intake and risk of cardiovascular disease. A review of a diet-disease relationship prepared for Food Standards Australia and New Zealand. Commissioned review, 2006.

86. Effect of a novel whey protein hydrolysate (NatraBoost XR) on recovery of muscular function following muscle damage induced by eccentric exercise. Report prepared for MG Nutritionals, 2006.

87. Effect of Vespa Amino Acid Mixture (VAAM) on fat oxidation during exercise, and exercise performance, in well-trained athletes. Report prepared for Meiji Dairies Corporation, 2006.

Manuscripts under preparation/review

1. Hill AM, JD Buckley, KJ Murphy, PRC Howe. Combining omega-3 supplementation with regular aerobic exercise to improve cardiovascular and metabolic health. *Under review* (Am J Clin Nutr).
2. Wycherley T, Buckley J, Clifton P, Noakes M, Brinkworth G. Weight loss with and without aerobic exercise improves cardiovascular disease risk factors but not flow-mediated dilatation in overweight and obese subjects with type 2 diabetes. *In preparation.*
3. Buckley J, Thomson R, Coates A, Rowney M, DeNichilo M, Howe PRC. Hydrolyzed whey protein isolate enhances recovery of muscle force-generating capacity following eccentric exercise. *In preparation.*
4. Buckley J. Alpha-cyclodextrin. In: Handbook of Fiber Ingredients. Taylor & Francis, CRC Press (contracted for publication in 2007).
5. Howe PRC, Mori T and J Buckley. The relationship between omega-3 fatty acid intake and risk of cardiovascular disease. A review of a diet-disease relationship. *In preparation.*
6. Buckley JD, S Burgess, K Murphy, A Coates, PRC Howe. Fish oil supplementation does not improve running performance or recovery in elite footballers. *In preparation.*
7. Hill AM, Worthley C, Murphy KJ, Buckley JD, Ferrante A, PRC Howe Omega-3 fatty acid supplementation and regular moderate exercise: differential effects of a combined intervention on neutrophil function. *In preparation*
8. Bourdon PC, Buckley JD. A single exercise test for assessing physiological and performance parameters in elite rowers: the two-in-one test. *In preparation.*
9. Davison K, Hill A, Howe PRC, Buckley JD. Exercise induced changes in the pulse waveform as an index of endothelium dependent vasodilation. *In preparation.*
10. Ninio DM, AM Hill, JH Smith, K Murphy, J Buckley, DA Saint, PRC Howe. Fish oil enhances heart rate variability in overweight adults. *In preparation.*
11. Saint D, AM Hill, J Buckley, PRC Howe. Dose-response effects of fish oil on heart rate variability in overweight adults. *In preparation.*

12. Halyburton A, Buckley J, Clifton P, Noakes M, Wilson C, Brinkworth G. Effects of a low-carbohydrate diet on mood and cognitive function. *In preparation.*
13. Kenyon R, Buckley J, Clifton P, Noakes M, Fenech M, Brinkworth G. Effects of a low-carbohydrate diet on markers of DNA damage. *In preparation.*
14. Brinkworth G, Clifton P, Noakes M, Fenech M, Buckley J. Effects of a low-carbohydrate diet on physical function and exercise capacity. *In preparation.*
15. Davison K, Bircher S, Hill A, Coates A, Buckley J. Fitness and fatness: effects on vascular reactivity and fat oxidation during exercise. *In preparation.*
16. Sioutis S, Coates A, Buckley J, Howe PRC. Regular consumption of omega-3 enriched pork increases docosahexaenoic acid incorporation into erythrocyte membranes. *In preparation.*
17. Milte C, Coates A, Buckley J, Howe PRC. DHA intake affects biomarkers of omega-3 status and cardiovascular risk. *In preparation.*
18. Tsiros M, Sinn N, Coates A, Buckley J, Howe PRC. Cognitive behavioural therapy for reducing obesity in adolescents: a review. *In preparation.*
19. Tsiros M, Sinn N, Coates A, Buckley J, Howe PRC. A program of cognitive behavioural therapy reduces obesity in adolescents. *In preparation.*
20. Buckley JD, S Graham. Overtraining in athletes: a review. *In preparation.*
21. Buckley JD, S Graham. Heart rate kinetics at the start of exercise predict exercise performance in junior athletes. *In preparation.*
22. Buckley JD, Brinkworth GD. Efficacy and safety of low carbohydrate diets. *In preparation.*
23. Thomson R, Brinkworth GD, Buckley JD. Diet and lifestyle for managing polycystic ovary syndrome: a review. *In preparation.*
24. Parker B, Coates A, Thorp A, Howe PRC, Buckley J. Collection of blood samples by venepuncture increases CCK: a warning for satiety research study design. *In preparation.*

Patents

Australian Patent (Patent Type AU-B2, Aust. Pat. No. 741299) entitled "A food composition and method of using same", for the use of bovine colostrum as a nutritional supplement for improving athletic performance, recovery and health.

Media coverage

Print Media

The Advertiser (Adelaide) – Wednesday July 19, 1995 – Elite program shows up highs and lows of sport. An article on research which showed that being tested at even moderate altitude reduces maximal oxygen uptake in elite cyclists.

The Advertiser (Adelaide) – August 4, 1998 – Fears of intense exercise damage. An article on research which showed that endurance exercise training can increase intestinal permeability.

The Australian (Front Page Article) – Friday October 9, 1998 – Nothing but the breast for champions. An article on the performance enhancing effects of bovine colostrum and its use by the Adelaide Crows Football Club in the lead up to their 1998 premiership win.

The Daily Yomiuri (Japan) – Saturday October 10, 1998 – Aussies rediscover virtues of milk. An article on the performance enhancing effects of bovine colostrum.

The Australian – Wednesday October 14, 1998 – Athletes spring back with colostrum. An article on the effects of bovine colostrum on recovery from prior exercise.

The Daily Observer (Canada) – Wednesday October 14, 1998 – Mother's milk may help athletes. An article on the effects of bovine colostrum on athletic performance.

The Sydney Morning Herald– Friday November 5, 1999 – A mother of a sports supplement. An article on the effects of bovine colostrum on athletic performance.

The Advertiser (Adelaide) – Friday November 12, 1999 – Milk bringing cream to the top, naturally. An article on the performance enhancing effects of bovine colostrum.

The Daily Mail (United Kingdom) – Wednesday November 15, 2005 – Is modern medicine just what the doctor ordered? An article on the benefits of colostrum for reducing the incidence of colds and flu.

Electronic Media

The 7.30 Report (ABC TV Current Affairs show) – Wednesday October 14, 1998 - 10 min segment on the performance enhancing effects of bovine colostrum.

All TV and Radio news services (Australian and International) – Wednesday October 14, 1998 – All TV and radio news services picked up stories released by AAP and Reuters on the performance enhancing effects of bovine colostrum and its use by athletes.

ABC Radio (National) – December 8, 2002 – Interview to discuss the effects of bovine colostrum on immune function.

Catalyst (ABC TV Science Show) – Thursday April 1, 2004 – 10 min segment on the performance enhancing effects of bovine colostrum and its use by athletes leading up to the 2004 Athens Olympic games.

Channel 7 news (National) – Thursday June 4, 2004 – news article on research on Omega-3 fatty acids and exercise effects on obesity

Life FM – Tuesday 17 August 2004 – interview regarding the effects of nutrition on athletic performance of olympic athletes.

Channel 7 news (National) – Tuesday 15 November 2005 – news article on research showing that taking fish oil during exercise can reduce body fat.

Channel 7 news – Monday 30 January 2006 – news article on research study in Whyalla which will introduce low glycemic index foods to reduce body fat.

Channel 10 news – Friday 3 February 2006 – news article on research study investigating effects of whey peptides on mood, satiety, muscle strength and recovery.

Radio Adelaide – Tuesday 7 February 2006 – interview on research study investigating effects of whey peptides on mood and satiety.

Radio Adelaide – Thursday 9 February 2006 – interview on research study investigating effects of whey peptides on muscle strength and recovery.

ABC News – Thursday 2 March 2006 – interview regarding role of whey protein isolate on muscle recovery.

Channel 7 news – Saturday 4 March 2006 – news article on study examining effects of supplementation with whey protein isolate on recovery of muscle function, performance and inflammation.

Channel 9 news – Wednesday 8 March 2006 – interview in relation to use of cognitive behavioural therapy for treatment of adolescent obesity.

Channel 2 Stateline Program – Friday 17 March 2006 – interview in relation to health benefits of cocoa.

Other

DEXA Licence Holder (Environmental Protection Agency) for the operation of a bone densitometer for research purposes.

Occupational Health Safety and Welfare courses completed in:

- Health and Safety Fundamentals
- Incident Investigation
- Manual Handling
- Hazard Management
- Injury Management for Managers and Supervisors

Occupational Health Safety and Welfare Management Leadership Program completed September 2005.

Biochem J. 1986 January 1; 233(1): 215–221.

Comparative binding of bovine, human and rat insulin-like growth factors to membrane receptors and to antibodies against human insulin-like growth factor-1.

L C Read, F J Ballard, G L Francis, R C Baxter, C J Bagley, and J C Wallace

Abstract

The immunological properties of human, bovine and rat insulin-like growth factors (IGF) and insulin were compared in competitive binding studies with Tr10 and NPA polyclonal antisera raised in rabbits against human IGF-1. ***Bovine IGF-1 was 11-19% as effective as human IGF-1 in competing for binding with 125I-labelled human IGF-1***, whereas IGF-2 reacted poorly and insulin did not compete. Similar competitive binding curves were obtained with the mouse monoclonal anti-(human IGF-1) antibody 3D1, except that bovine IGF-1 showed a severalfold greater affinity for the monoclonal antibody than for either polyclonal antiserum. Membranes isolated from human placenta, sheep placenta and foetal-human liver were used as sources of cellular receptors. In human placental membranes, most of the binding of IGF-1 tracers could be attributed to a type-1 receptor, because insulin inhibited up to 65% of tracer binding. The other two tissues apparently contain only type-2 receptors, as evidenced by the very low potency of bovine or human IGF-1 in competing for binding with IGF-2 tracers and the absence of any competition by insulin. In competition for binding with labelled bovine or human IGF-1 to human placental membranes, bovine IGF-1 had a similar potency to human IGF-1, whereas bovine IGF-1 was more potent in binding studies with tissues rich in type-2 receptors. Rat IGF-2 was considerably less effective than human IGF-2 in competition for receptors on any of the membrane preparations.

38, 173 (1979); B. A. Hills, *Thorax* 36, 1 (1981); B. A. Hills, *J. Physiol. (London)* 325, 175 (1982).
4. W. M. Linfield, in *Cationic Surfactants*, J. Jungermann, Ed. (Dekker, New York, 1969), p. 53.
5. H. Davenport, *Sci. Am.* 226, 86 (January 1972).
6. ———, *Gastroenterology* 46, 245 (1964); ———, H. A. Warner, C. F. Code, *ibid.* 47, 142 (1964); H. W. Davenport, *ibid.* 59, 505 (1970).
7. H. W. Davenport, *Gut* 6, 513 (1965).
8. B. A. Hills, B. D. Butler, L. M. Lichtenberger, *Am. J. Physiol.*, in press.
9. A. Slomiany, S. Yano, B. L. Slomiany, G. B. J. Glass, *J. Biol. Chem.* 253, 3785 (1978); M. K. Wassef, Y. N. Lin, M. I. Horowitz, *Biochim. Biophys. Acta* 573, 222 (1979); L. M. Lichtenberger, B. D. Butler, B. A. Hills, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 41, 1124 (1982).
10. A. Robert, J. E. Nezamis, C. Lancaster, A. J. Hanchar, *Gastroenterology* 77, 433 (1979).
11. W. H. Crosby and F. W. Furth, *Blood* 11, 380 (1956).
12. The benzidine analysis was performed by incubating 25 μl of the sample (gastric aspirate or hemoglobin standard) in a tube containing 0.5 ml of benzidine reagent (1 percent benzidine dihydrochloride in acetic acid) and 0.5 ml of 1 percent H_2O_2 at 25°C. After 20 minutes 5 ml of 10 percent acetic acid was added to the solution and the incubation was continued for an additional 10 minutes. Absorbance of the colored reaction product was then measured spectrophotometrically at a wavelength of 515 nm.
13. A. Robert, J. E. Nezamis, J. P. Phillips, *Gastroenterology* 55, 481 (1968); M. M. Cohen, *Lancet* 1978-II, 1253 (1978); C. Johansson, B. Kollberg, R. Nordeman, S. Bergstrom, *ibid.* 1979-I, 317 (1979); A. Robert, in *Physiology of the Gastrointestinal Tract*; L. R. Johnson, Ed. (Raven, New York, 1981), vol. 2, p. 1407.
14. G. Colaccico, M. K. Basu, A. K. Ray, M. Wittner, R. M. Rosenbaum, *Prostaglandins* 14, 283 (1977); G. G. Anderson, J. A. Cidlowski, P. M. Absher, J. R. Hewitt, W. H. J. Douglas, *ibid.* 16, 923 (1978).
15. F. A. Fitzpatrick and M. A. Wynalda, *ibid.* 12, 1037 (1976); J. R. Vane, *Nature (London) New Biol.* 231, 232 (1971).
16. U. Skipski, R. F. Peterson, J. Sanders, M. Barday, *J. Lipid Res.* 4, 227 (1963).
17. G. Rouser, A. N. Siarotos, S. Fleisher, *Lipids* 1, 85 (1966).
18. R. J. Mason, J. Nellenbogen, J. A. Clements, *J. Lipid Res.* 17, 281 (1976).
19. Rat oxyntic mucosa was extracted in accordance with the method of Folch *et al.* (*20*), oxidized with OsO_4, and applied to a small column containing 0.8 g of neutral alumina. After 10 ml of chloroform and methanol (20:1) were added to the columns, the dipalmitoyl phosphatidylcholine fraction was eluted with 5 ml of chloroform, methanol, and 7*M* NH_4OH (70:30:2) and quantitated by phosphorus analysis (*17*, *18*). The concentration of oxyntic dipalmitoyl phosphatidylcholine is expressed as micrograms per gram of tissue (wet weight).
20. J. Folch, M. Lees, G. H. Sloane-Stanley, *J. Biol. Chem.* 226, 497 (1957).
21. C. A. Owen, Jr., J. L. Bollman, J. H. Grindlay, *J. Lab. Clin. Med.* 44, 238 (1954).
22. We thank A. Robert and J. Pike for donating PGE_2.

7 September 1982; revised 9 November 1982

Polypeptide Transforming Growth Factors Isolated from Bovine Sources and Used for Wound Healing in vivo

Abstract. *Transforming growth factors, which are polypeptides that induce the transformed phenotype in nonneoplastic cells, have been isolated in bulk amounts from bovine salivary gland and kidney. In experiments in which wound healing chambers were implanted subcutaneously in the backs of rats, these bovine transforming growth factors accelerated the accumulation of total protein, collagen, and DNA in treated chambers. These studies thus show an effect of an isolated transforming growth factor in vivo.*

Although many new peptide growth factors have been isolated and characterized (*1*), there have been few studies on the activity of these materials in vivo. An important area for potential application of peptide growth factors is the enhancement of wound healing. Despite the need for rapid healing in the treatment of severe burns, trauma, diabetic and decubitus ulcers, and other conditions, there is no practical way at present to accelerate wound healing with pharmacological agents. Although it has been suggested that epidermal growth factor (EGF) might be of benefit (*2*), it has not yet been extensively used in a practical way for wound healing. The ability of a related and newly discovered set of polypeptides, the transforming growth factors (TGF's), to promote growth of cells under highly restrictive conditions in vitro suggests that TGF's might have useful applications in vivo for wound healing. We now report a large-scale isolation of TGF's from readily available bovine sources and a demonstration of the in vivo activity of an isolated TGF in an experimental wound healing system.

Transforming growth factors are a heterogeneous set of low molecular weight polypeptides defined by their ability to induce the transformed phenotype—particularly anchorage-independent growth in soft agar—in untransformed indicator cells that ordinarily do not grow in soft agar (*3*, *4*). Transforming growth factors have been found in almost all tissues, both nonneoplastic and neoplastic, from many different species of animals, including man (*4*, *5*). The finding of TGF's in blood platelets (*6*) suggests that TGF's may have a role in wound healing and tissue repair. Since TGF's have important functional interactions with EGF and its receptor, we have proposed a new classification of TGF's based on their relationships with EGF (*7*). Type α TGF's are those that compete with EGF for receptor binding and do not require EGF for induction of colony formation by indicator cells in soft agar, whereas type β TGF's do not compete with EGF for receptor binding, but do require the presence of EGF (or EGF-like polypeptides) for induction of colony formation in soft agar. Both types of TGF activity have been isolated from both neoplastic and nonneoplastic cells and tissues (*3*–*8*).

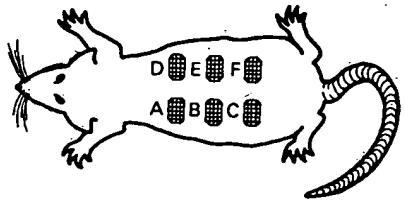

Fig. 1. Diagram of arrangement of wound chambers in the back of a rat. The chambers are made of stainless steel wire mesh and are 2 cm long and 1 cm in diameter. Male Buffalo rats, weighing 400 to 500 g and approximately 12 months old, were used in all experiments.

Full details of purification of bovine β-TGF's will be reported elsewhere. Briefly, salivary glands or kidneys, obtained fresh from the slaughterhouse and frozen immediately on dry ice, were extracted in 2-kg portions with acidified ethanol (*8*). Extracts from 6 to 8 kg of tissue were combined and chromatographed on Bio-Gel P-30 with 1*M* acetic acid on an 80-liter bed volume column (*9*). Most of the in vivo studies reported below were done with salivary gland or kidney TGF's purified to this stage; their activity in vitro was enhanced approximately 20-fold by the presence of 2 to 5 ng of EGF per milliliter in the assay. After chromatography on Bio-Gel P-30, the bovine β-TGF's were purified further by high-performance liquid chromatography (HPLC) on μBondapak C_{18} columns for which an acetonitrile gradient in 0.1 percent trifluoroacetic acid was used; this was followed by a second HPLC step on μBondapak CN columns with a gradient of *n*-propanol in 0.1 percent trifluoroacetic acid (*10*).

Activity of isolated salivary gland and kidney β-TGF's in vivo was measured in a standard experimental wound healing model. Six, empty wire mesh wound chambers (Schilling-Hunt) (*11*) were surgically inserted subcutaneously in the back of rats in a symmetrically paired fashion (pairs A and D, B and E, and C and F in Fig. 1). The animals respond to these chambers as if they were wounds, and eventually the chambers become filled with fibroblasts and collagen. By the fourth day after insertion, the chambers become encapsulated with connective tissue; but there are few cells within the chambers themselves. There is thus a defined, enclosed space within the chambers where a wound healing response can be quantitatively measured. At this time, daily injections of TGF (0.1 ml in sterile, phosphate-buffered saline) into

chambers A, B, and C were begun. Except where noted, a low level of murine EGF (*12*) was included in the TGF injections to potentiate TGF activity. Chambers D, E, and F were used as controls and were injected with an amount of bovine serum albumin (BSA), either alone or in combination with either TGF or EGF, to provide an amount of total protein equivalent to the amount of TGF injected into chambers A, B, and C. Injections were made once daily for either 5 days (Table 1) or 9 days (Table 2). All injected materials were sterile. The rats were killed 6 hours after the last TGF injection; the animals treated for 9 days (Table 2) were given intraperitoneal injections of [^{3}H]thymidine (0.5 mCi; specific activity, 6.7 Ci/mmole), with the last TGF injection. The chambers were removed from the rats, all connective tissue on the outside of the wire mesh was peeled away, and the contents of each chamber were determined.

Table 1 shows that 5 days of treatment with β-TGF from either bovine salivary gland or kidney caused a highly significant increase in total protein in the treated chambers, as compared to control chambers treated with an equivalent amount of BSA (experiments 1 and 3). The salivary gland TGF was still highly active after two steps of purification by HPLC (experiment 2). The effects observed are not the sole result of the minute amounts of EGF that had been used to potentiate the activity of β-TGF, since a highly significant difference between treated chambers A, B, and C, compared with control chambers D, E, and F was still observed when EGF was used as the control substance (experiment 4). Furthermore, when all chambers were treated with TGF, and only A, B, and C were treated with EGF, no significant difference was observed (experiment 5). At the end of experiments 1 to 4, we consistently observed that chambers A, B, and C were more firmly fixed in the surrounding connective tissue than the respective matched control chambers; this suggested that the effects of TGF were also manifested in the area immediately surrounding the chambers.

To measure the effects of bovine salivary TGF on DNA and collagen content of the chambers, we found it necessary to treat the animals for longer than 5 days. In an experiment in which 13 rats were treated for 9 days (Table 2), the increases in total protein, total DNA, thymidine incorporation into DNA, and total collagen were highly significant. Histological examination of the contents of chambers treated with TGF confirmed the occurrence of fibroblastic proliferation and formation of collagen. A sterile infiltrate of inflammatory cells, which are known to be involved in physiological wound healing (*13*), was also found within both treated and control chambers.

The results obtained in both experiments indicate that β-TGF's can significantly accelerate a wound healing response. Further studies in other wound healing models, such as measurement of effects on tensile strength of linear wounds and rate of healing of granulating wounds, are needed. The new growth factors that we have isolated are biologically active at the nanogram level in vitro and in vivo and can be obtained on a scale large enough for further investigation of their intrinsic physiological role or possible therapeutic application.

Table 1. Wound healing response to bovine salivary gland or kidney TGF after 5 days of treatment. Transforming growth factors were prepared and injected as described in the text. Each dose contained 25 times the amount of TGF found optimal for colony formation by normal rat kidney cells in a standard soft agar assay (*4*) and ranged from 18 to 42 colony-forming units (*9*) per dose. The amounts of protein injected per dose were: 7 μg in experiments 1, 4, and 5; 25 μg in experiment 3, and 0.7 μg in experiment 2. All doses of EGF were 20 ng. Total protein in wound chambers was measured by the method of Lowry *et al.* (*14*). Statistical analysis of the data was made by comparison of matched pairs of the chambers (A versus D, B versus E, and C versus F) shown in Fig. 1.

Experiment	Treatment		Number of matched pairs of chambers	Average amount of protein per chamber (mg)*		Average ratio†	*P*‡
	Chambers A, B, C	Chambers D, E, F		A, B, C	D, E, F		
1	TGF (salivary, P-30) + EGF	BSA	36	10	3.9	3.8 ± 0.6	< .001
2	TGF (salivary, HPLC) + EGF	BSA	9	8.4	2.9	4.6 ± 1.0	< .02
3	TGF (kidney, P-30) + EGF	BSA	9	8.1	3.5	5.2 ± 1.5	< .005
4	TGF (salivary, P-30) + EGF	EGF	9	9.6	5.3	2.1 ± 0.3	< .02
5	TGF (salivary, P-30) + EGF	TGF	9	11.2	9.6	1.4 ± 0.3	.5

*For method of computation, see (*15*). †Average of matched-pair ratios, A/D, B/E, C/F, ± standard error of the mean (S.E.M.). ‡One-sided *P* values based on the sign test.

Table 2. Wound healing response to bovine salivary gland TGF after 9 days of treatment. Chambers A, B, and C were injected once daily with 7 μg of TGF (P-30) plus 20 ng of EGF. Chambers D, E, and F were provided an equal amount of BSA.

Measurement	Number of matched pairs of chambers	Average content per chamber		Average ratio†	*P*‡
		A, B, C	D, E, F		
Protein (mg)	30	24	15	1.6 ± 0.05	< .001
DNA (μg)	30	21	8.6	2.6 ± 0.16	< .001
[^{3}H]Thymidine (counts per minute per microgram of DNA)	30	45	30	1.7 ± 0.09	< .001
Collagen (mg)	9	5.2	3.2	1.8 ± 0.2	< .005

*Measurements were made as discussed in (*16*). †Average of matched-pair ratios, A/D, B/E, C/F, ± S.E.M. ‡One-sided *P* values based on the sign test.

MICHAEL B. SPORN
ANITA B. ROBERTS, JAMES H. SHULL
JOSEPH M. SMITH, JERROLD M. WARD
National Cancer Institute, Bethesda, Maryland 20205

JARO SODEK
National Institute of Dental Research, Bethesda, Maryland 20205

References and Notes

1. R. Baserga, Ed., *Tissue Growth Factors* (Springer-Verlag, New York, 1981).
2. G. Carpenter, in *ibid.*, p. 123.
3. J. E. De Larco and G. J. Todaro, *Proc. Natl. Acad. Sci. U.S.A.* 75, 4001 (1978).
4. A. B. Roberts, M. A. Anzano, L. C. Lamb, M. B. Sporn, *ibid.* 78, 5339 (1981).
5. G. J. Todaro, C. Fryling, J. E. De Larco, *ibid.* 77, 5258 (1980); B. Ozanne, R. J. Fulton, P. L. Kaplan, *J. Cell. Physiol.* 105, 163 (1980); H. L. Moses, E. L. Branum, J. A. Proper, R. A. Robinson, *Cancer Res.* 41, 2842 (1981); J. A. Proper, C. L. Bjornson, H. L. Moses, *J. Cell. Physiol.* 110, 169 (1982); D. R. Twardzik *et al.*, *Cancer Res.* 42, 590 (1982).
6. C. B. Childs, J. A. Proper, R. F. Tucker, H. L. Moses, *Proc. Natl. Acad. Sci. U.S.A.* 79, 5312 (1982).
7. A. B. Roberts, C. A. Frolik, M. A. Anzano, M. B. Sporn, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, in press; M. A. Anzano, A. B. Roberts, C. A. Meyers, A. Komoriya, L. C. Lamb, J. M. Smith, M. B. Sporn, *Can. Res.* 42, 4776 (1982).
8. A. B. Roberts, L. C. Lamb, D. L. Newton, M. B. Sporn, J. E. De Larco, G. J. Todaro, *Proc. Natl. Acad. Sci. U.S.A.* 77, 3494 (1980).
9. TGF activity, assayed as described (*4*), eluted in a broad peak between ribonuclease (13,700) and

insulin (5700) markers. At this stage of purification, the TGF's had a specific activity approximately 10- to 25-fold higher than that of the acidified ethanol extracts, with a range of recovery of 150,000 to 200,000 colony-forming units per kilogram of tissue. A colony-forming unit is defined as the amount of TGF that will induce the formation of 1000 colonies of normal rat kidney cells > 3100 μm^2 under standard assay conditions (*4*), in the presence of EGF (5 ng/ml).

10. M. A. Anzano, A. B. Roberts, L. C. Lamb, J. M. Smith, M. B. Sporn, *Anal. Biochem.* **125**, 217 (1982). After the two HPLC steps, each of the bovine β-TGF's had an absolute requirement for EGF for colony-forming activity. The yield of HPLC-purified TGF was approximately 20 to 100 μg per kilogram of tissue, with a total activity of 7000 to 18,000 colony-forming units.
11. T. K. Hunt, P. Twomey, B. Zederfeldt, J. E. Dunphy, *Am. J. Surg.* **114**, 302 (1967).
12. Prepared as in (*4*), which is a modification of the procedure of C. R. Savage and S. Cohen, *J. Biol. Chem.* **247**, 7609 (1972).
13. R. Ross, *Biol. Rev.* **43**, 51 (1968).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
15. In all experiments, chambers were inserted in several untreated rats and then removed on the 4th day, at the time when injections of TGF's were begun. Total protein was determined for these chambers, and these "zero-time" values were subtracted from the respective values obtained from rats treated with TGF or control materials. Comparison of the ratios (A/D, B/E, C/F) for the zero-time values (a total set of 33 matched pairs of chambers) showed no significant difference between the left and right side of the rat.
16. Protein was determined as in (*14*). [^{3}H]Thymidine incorporation and total DNA were determined on portions of tissue that had been dissolved in 1*M* NaOH, precipitated with ice-cold 0.3*M* perchloric acid (PCA), washed with ice-cold 0.2*M* PCA, and finally extracted in 0.5*M* PCA at 70°C. Portions of the final extract were used for determination of radioactive counts in a liquid scintillation counter, and deoxyribose was determined by the method of K. Burton [*Biochem. J.* **62**, 315 (1956)]. Collagen was determined as hydroxyproline after hydrolysis with 6*M* HCl. Typing of the collagen by gel electrophoresis of pepsin digests showed no difference between treated and control chambers; Type I collagen was the predominant form.
17. We thank G. Martin and M. Anzano for helpful suggestions, L. Lamb for assistance with the determinations, C. Brown for advice on the statistical analysis, and R. Morsillo for manuscript preparation.

23 August 1982

Demonstration of a Saturable Binding Site for Thyrotropin in *Yersinia enterocolitica*

Abstract. *Several lines of evidence suggest that there might be immunologic cross-reactivity between the thyroid plasma membrane in humans and antigenic determinants in the enteric pathogen* Yersinia enterocolitica. *Studies were therefore performed to determine whether* Y. enterocolitica, *like the thyroid membrane, contains a thyrotropin binding site. A saturable binding site for bovine thyrotropin was indeed demonstrable, particularly in preparations of the organism that have been treated with ethylenediaminetetraacetate and lysozyme. Hormonal specificity of the binding site, as judged from the inhibition of binding of ^{125}I-labeled bovine thyrotropin, was similar to that of the thyrotropin receptor in human thyroid tissue.*

In this report, we describe the presence of a specific, saturable binding site for the mammalian peptide hormone thyrotropin (TSH) in the pathogenic Gram-negative bacillus *Yersinia enterocolitica*. Several groups of observations prompted us to explore this question. First, patients infected with *Y. enterocolitica* commonly display several disorders thought to be autoimmune in nature, including arthritis, erythrema nodosum, Reiter's syndrome, and iritis (*1*). Further, the serum of such patients contains a variety of antibodies to epithelium (*2*), including some which by immunofluorescent techniques can be shown to bind to the cytoplasm and plasma membrane of human thyroid epithelium (*3*). The second group of observations relates to patients with the autoimmune thyroid diseases, Graves' disease and Hashimoto's disease; serum from these patients contains autoantibodies against a variety of thyroid antigens, including thyroid microsomal antigens and thyroglobulin (*4*). It is currently thought that among these antibodies in Graves' disease is an antibody against the TSH receptor which, like TSH, binds to the thyroid membrane, activates adenylate cyclase therein, and initiates the thyroid hyperfunction characteristic of this disorder (*4*). It seemed of particular interest, therefore, that patients with Graves' disease and Hashimoto's disease display, with inordinate frequency, both circulating agglutinins against *Y. enterocolitica* and evidence of cell-mediated immunity against this organism (*5*). Also of relevance is a report that indicates that rabbits immunized against human or rabbit thyroid tissue develop antibodies that are reactive against *Y. enterocolitica* (*6*).

Together these findings raise the possibility that one or more components of the thyroid membrane and of *Y. enterocolitica* share common or cross-reacting antigenic determinants. To explore this question, we looked for functional homology between *Y. enterocolitica* and the thyroid plasma membrane, reasoning that this might reflect structural similarities that could, in turn, account for immunologic cross-reactivity. The best characterized functional unit of the thyroid membrane is its receptor for TSH, the properties of which have been studied by well-established techniques (*7*). Therefore, we conducted experiments to determine whether *Y. enterocolitica* could be shown to contain any site functionally analogous to the TSH receptor in thyroid plasma membranes, and such indeed proved to be the case.

Highly purified bovine TSH (bTSH), labeled with ^{125}I by a stoichiometric chloramine-T method (*8*), was used as a probe. Cultures of *Y. enterocolitica*, serological type 0:3, preserved in the frozen state, were provided by one of us (S.W.). For each experiment, frozen organisms were thawed out and cultured on blood agar plates, and were then subcultured overnight at room temperature in brain-heart infusion broth.

Initial experiments were directed at determining whether saturable binding of ^{125}I-labeled bTSH to the *Yersinia* organism could be demonstrated. Organisms were harvested by centrifugation and washed once with 10 m*M* tris-HCl buffer, *p*H 7.4. Cells were either studied live, killed by treatment with heat or 0.4 percent Formalin, or treated with lysozyme or lysozyme-EDTA, according to techniques previously described (*9*). Various concentrations of cells were then suspended in 10 m*M* tris-HCl buffer, *p*H 7.4, containing ^{125}I-labeled bTSH (approximately 10^{-11}*M*) (*10*). Some tubes contained, in addition, 1.0 U of unlabeled TSH for measurement of nonsaturable or nonspecific binding. Suspensions were incubated for 60 minutes at room temperature, after which the bacterial residue was sedimented and counted. Under these conditions, nonspecific binding was regularly in the range of 1 to 2 percent of added radioactive bTSH. In all preparations, binding of ^{125}I-labeled bTSH in excess of nonsaturable binding was detected and remained constant for at least 6 hours. This specific binding varied in amount with the content of bacterial protein and the manner in which the bacteria had been treated. The least binding was evident in live and lysozyme-treated bacteria, and greatest binding in bacteria treated with a combination of lysozyme and EDTA, in which preparations more than 20 percent of added ^{125}I-labeled bTSH was bound (Fig. 1). We assume that the differences in tracer binding among bacteria prepared by the various methods were the result of differences in the ease of access of the tracer to the binding site. Because the greatest binding of ^{125}I-labeled bTSH was observed in lysozyme-EDTA preparations, this mode of treating the organisms was used in all subsequent studies.

Characteristics of the saturable binding were studied in comparable experi-